МИКРОБИОЛОГИЯ

A Translation Of

MICROBIOLOGY

(MIKROBIOLOGIYA)

(Russian Original Dated May-June, 1961)

UNIVERSITY OF ILLINOIS
BIOLOGY
JAN 25 1962
CHICAGO

Published by
THE AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES
2000 P Street, N.W.
Washington 6, D. C.

A Translation of

MICROBIOLOGY

(Mikrobiologiya)

Dr. J. R. Porter, Advisory Editor

AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES

OFFICERS

Tracy M. Sonneborn
President

Bernard S. Meyer
Vice President

F. P. Cullinan
Secretary-Treasurer

Hiden T. Cox
Executive Director

GOVERNING BOARD

American Academy of Microbiology
Roger Reid

American Bryological Society
William C. Steere

American Physiological Society
Wallace O. Fenn

American Phytopathological Society
George L. McNew

American Society for Horticultural Science
Freeman S. Howlett

American Society of Human Genetics
Karl A. Stiles

American Society of Ichthyologists and Herpetologists
R. G. Zweifel

American Society of Naturalists
Albert P. Blair

American Society of Parasitologists
A. C. Walton

American Society of Plant Physiologists
Bernard S. Meyer

American Society of Plant Taxonomists
Reed C. Rollins

American Society of Zoologists
W. Gardner Lynn

Botanical Society of America
Paul J. Kramer

Conference of Biological Editors
Fred R. Cagle

Ecological Society of America
David E. Davis

Entomological Society of America
F. W. Poos

Genetics Society of America
Carl P. Swanson

Mycological Society of America
John A. Stevenson

National Association of Biology Teachers
Dorothy Matala

Phi Sigma Society
Fred S. Orcutt

Poultry Science Association
A. M. Guhl

Society for Industrial Microbiology
E. L. Dulaney

Society of General Physiologists
Kenneth Thimann

Society for the Study of Development and Growth
Clifford Grobstein

The translation and publication of this and several other Russian language biological journals is a project of the American Institute of Biological Sciences. This project is financed largely by a grant from the National Science Foundation. It is intended that each journal be a literal translation of all scientific material in the Soviet journal and the contents, therefore, are those of the original authors. The American Institute of Biological Sciences does not edit or otherwise determine the contents of the translated material.

The volumes in English translation bear the Soviet volume and issue numbers. They appear in the same number of issues per year as the original Soviet journals.

Yearly Subscriptions: $20.00, individuals and industrial libraries (U.S. and Canada); $15.00, AIBS members and all other libraries; $2.50, additional to each price, foreign. Single copies: $4.00.

Subscriptions should be addressed to the American Institute of Biological Sciences,

2000 P Street, N.W., Washington 6, D. C.

Copyright © 1961 by the American Institute of Biological Sciences

MICROBIOLOGY

A translation of Mikrobiologiya

(Russian Original Dated May-June, 1961)

Vol. 30, No. 3, pp. 337-488 November-December, 1961

CONTENTS

	PAGE	RUSS. PAGE
An Autotrophic Bacterium Oxidizing Formic Acid. Yu. I. Sorokin	337	385
Symbiotic Culture of a New Microorganism Oxidizing Manganese. G. A. Zavarzin	343	393
A Contribution to the Species Composition of Actinomyces of Certain Districts of Southern China. T. S. Maksimova, T. P. Preobrazhenskaya, E. S. Kudrina, and M. A. Sveshnikova	346	396
Morphological Peculiarities of the Actinomycetes Actinomyces lavendulae and Actinomyces aureofaciens under Prolonged Cultivation in a Continuous Culture. M. N. Bekhtereva and I. G. Kolesnikova	351	402
Reflection of Plane-Polarized Light by Cells of the Aerial Mycelium of Actinomyces violaceus 829. L. V. Kalakutskii	358	409
Phage of the Thermophilic Micromonospora vulgaris. N. S. Agre	362	414
Effect of Aeration on Heterofermentative Lactic Acid Fermentation with Respect to Acidity of the Medium. N. N. Grechushkina	365	418
Relationship of Some Species of Gaseous Hydrocarbon-Oxidizing Bacteria to Hydrocarbons of the Paraffin Series. Z. P. Telegina	370	426
Dynamics of the Bacterial Microflora Growth in Chlorella Cultures. M. G. Vladimirova.	374	431
Some Direct Effects of Ionizing Radiations on the Yeast Cell. K. Vas and I. Farkas	378	436
Possibility of Ultrasonic Production of Non-Lysogenic Variants from Lysogenic Actinomyces Cultures. Ya. I. Rautenshtein, N. P. Fadeeva, and I. E. El'piner	381	441
Influence of Some Amino Acids on the Growth and Acid Production of the Aspergillus niger T_1 Mutant. I. D. Kasatkina	387	447
Ribonucleic Acids of Thermophilic and Mesophilic Cultures of Saccharomyces cerevisiae Strain XII. T.N. Evreinova, I. V. Marchenko, and L. G. Loginova	391	453
Transformation in Nodule Bacteria. R. Balassa and M. Gabor	396	457
Interspecies Transformation in Microorganisms. A. A. Imshenetskii and S. É. Mansurova	401	464
Effect of Disinfectants on Bacterial Cell Morphology and Biology. A. M. Malchenkov	403	466
Physiological Differences between Strains of Root Nodule Bacteria of Pea and Vetch Differing in Nitrogen-Fixing Activity. M. V. Fedorov and L. Nitsé	408	473
Adjustment of Various Azotobacter Strains on the Roots and Rhizosphere of Spring Wheat Grown on a Podzol Soil. L. I. Vigorov	412	478
Resistance of Sugar Beet Rhizosphere Bacteria to Antibiotics. P. Gyurkó, M. Kecskés, and E. Manninger	416	484
Efficacy of Peat–Manure Composts with Reference to Development of Butyric Acid Bacteria in Them. S. S. Abyzov	419	489
Effect of Herbicides on Soil Microflora. I. A. Geller and E. G. Khariton	423	494

CONTENTS (continued)

	PAGE	RUSS. PAGE
Microbiological Studies of Carpathian Sulfur Deposits. V. Occurrence of Sulfate-Reducing Bacteria in Sedimentary Rocks Composing Sulfur Deposits. M. V. Ivanov	428	500
Occurrence of Thiobacillus thioparus in Hydrogen Sulfide Underground Waters. G. A. Sokolova	431	503
Role of Microorganisms in Iron Corrosion in Water. V. V. Kuznetsov and L. V. Verzhbitskaya	436	511
Microbial Indicators of Organic Pollution of Waters by Industrial Effluents. I. Filamentous Bacteria of the Genus Cladothrix (Methods, Morphology, and Relation to Physical Environmental Factors). A. S. Razumov	439	515
Intensification of Biological Purification of Industrial Wastes. II. Microbiological Characterization of Activated Sludge Used to Purify Effluents Containing Hydrogen Sulfide. Ts. I. Rogovskaya and M. F. Lazareva	446	525
Purification of Microbial Histidine Decarboxylase. S. R. Mardashev and V. V. Mamaeva	450	530
Production of Purified Enzyme Preparations from the Surface of Mold Cultures. R. V. Feniksova and G. A. Molodova	453	534
Effect of Wheat Bran Quality on the Amylolytic Activity of Enzyme Preparations. T. I. Trainina, D. B. Lifshits, and E. Ya. Kalashnikov	457	540
Microbiology in Cairo. A. A. Imshenetskii	461	545
SURVEY		
Ecology of Nitrosomonas. O. M. Ul'yanova	464	550
BOOK REVIEWS		
The Russian Microbiologists S. N. Winogradsky and V. L. Omelyanskii. Reviewed by A. A. Imshenetskii	475	565
Fysiologie a Biochemie Mikrobu. Jirí Stárka. Reviewed by N. D. Ierusalimskii	477	566
CURRENT EVENTS		
Problems in Microbiology at the XV International Congress of the Milk Industry in London. A. Skorodumova	479	568
The 60th Anniversary of E. N. Mishustin	483	571
Samuil L'vovich Sobol'. A. I. Metelkin	485	573

ERRATA

Vol. 30, No. 2

Page	Location	Reads	Should read
272	Right-hand column, 4th line from bottom	... indicator ...	... China blue indicator ...
276	Left-hand column, 2nd line from top	... 1.5 times more ...	... 11 times more ...

AN AUTOTROPHIC BACTERIUM OXIDIZING FORMIC ACID

Yu. I. Sorokin

Borok Research Institute of Reservoir Biology, Academy of Sciences, USSR
Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 385-392, May-June, 1961
Original article submitted January 16, 1961

In the course of radiocarbon studies of CO_2 carbon assimilation by different groups of bacteria we encountered a curious phenomenon. We found that an aerobic bacterium which we isolated from mud synthesized its cellular substance from CO_2 carbon, i.e., it metabolized like an autotroph when grown on a medium containing formic acid. Below we give the results of experiments and a description of the diagnostic characters of this organism, which we describe as a new species of bacterium.

METHODS AND RESULTS

To obtain enriched cultures of bacteria growing on formic acid we used a buffered synthetic medium containing 5 g of sodium formate per liter as an oxidizable substrate.

Flasks containing the medium were inoculated with mud from Rybinsk Reservoir. After a month the medium in one of the flasks became turbid. With further subculturing in the same medium the growth of the culture became more vigorous. Three or four days after inoculation the medium became very turbid and was alkalized, despite its considerable buffering. After several subcultures the culture was plated out from dilutions onto agar medium containing sodium formate. From the colonies we obtained a pure culture which grew well on defined media containing formate, acetate, lactate, ethyl alcohol, or glucose.

With this culture we carried out experiments to reveal the part played by CO_2 in biosynthesis in this organism. We carried out similar experiments with a culture of a sulfate-reducing bacterium (Vibrio desulfuricans) and with a number of typical heterotrophic bacteria (Escherichia coli, Bacillus mesentericus, Clostridium acetobutylicum). During these experiments, in which C^{14} was used, we found that heterotrophic bacteria growing on glucose obtain from CO_2 6-8% of the carbon used for biosynthesis. As distinct from these bacteria, the facultative autotroph Vibrio desulfuricans, growing in a medium with lactic acid, obtains from CO_2 about 30% of the carbon required for biosynthesis (Sorokin [1960a]). Tomlinson and Barker [1954] showed that an anaerobic bacterium oxidizing ethyl alcohol in the presence of acetic acid (Clostridium kluyveri) synthesizes 25% of its body substance from CO_2 carbon. Thus, the available information indicates the important role of CO_2 in biosynthesis in some bacteria oxidizing low-molecular compounds.

In view of this it was of interest to study the role of CO_2 in biosynthesis in bacteria oxidizing formic acid during their growth on different carbon sources.

To discover the part played by CO_2 carbon in biosynthesis in these bacteria we used a radiocarbon method similar to that which we used to determine the isotopic effect in chemosynthesis in hydrogen bacteria (Sorokin [1960b]). For cultivation of the bacteria we prepared a medium of the following composition: H_3PO_4 (conc.) – 10 g; $MgSO_4$ – 0.1 g and NH_4Cl – 0.1 g; distilled water up to 1 liter. After sterilizing the medium we added the appropriate carbon source, the organic acids being added in the form of acids, and not as salts. The medium was then neutralized with 10% Na_2CO_3 solution to pH=7.2. This method of preparing the medium ensured maximal buffering and the highest content of HCO_3^- and CO_2 carbon for the least amount of added salts. A sterile solution of radioactive carbonate $Na_2C^{14}O_3$ was added to the neutral medium to give an activity of about $1 \cdot 10^6$ counts per liter. The experiments were performed in 3-liter bottles or flasks. The flasks were half-filled with medium and inoculated with a small quantity of culture. Some of the medium (500 ml) was then poured off for a control determination of the organic matter introduced on inoculation or during preparation of the medium, and also for a determination of the initial specific activity R_1 of the CO_2 and HCO_3^- carbon. The flasks were then sealed and incubated at 30 C. A considerable bacterial mass, in the form of a homogeneous suspension, usually accumulated within a week. At the end of the experiment we determined the final specific activity (R_2) of the CO_2 and HCO_3^- carbon and the specific activity of the carbon of the bacterial bodies by the method described in a previous paper (Sorokin [1960a,b]).

The bacterial bodies were separated from the medium by precipitation with aluminum hydroxide at pH 5.2. The culture was heated to boiling, acidified, and 10-15 ml of a 10% solution of $Al_2(SO_4)_3$ was added to

it. When the aluminum hydroxide deposit had settled the liquid was poured off. The deposit was concentrated by centrifugation and was dissolved in sulfuric acid. The obtained pure suspension of bacteria precipitated by the hydroxide was again centrifuged. In the bacterial deposit we determined the specific activity of the carbon of organic matter (r). The percentage of carbon derived from CO_2 (P) in the organic matter of the bacterial bodies was calculated from the percentage ratio of the specific activity of bacterial body carbon (r) to the mean specific activity of $CO_2 + HCO_3$-carbon of the medium from the formula: $P = \frac{2r \cdot 100 \cdot 1,06}{R_1 + R_2}$ %.

The factor 1.06 is the correction for the isotopic effect – the slower assimilation of $C^{14}O_2$ in comparison with $C^{12}O_2$ (Sorokin [1960b]).

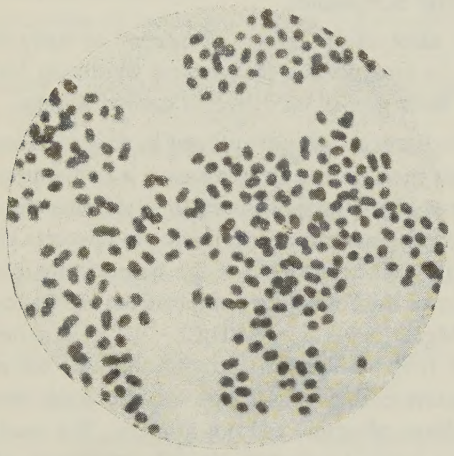


Fig. 1. Smear from young culture. 2000 ×.

The determination of the relative amount of CO_2 carbon assimilated by the bacteria during their growth on organic acids is perfectly reliable if there are no exchange reactions between CO_2 and the carboxyl groups of the particular acids. Numerous experiments by various authors who have used labeled CO_2 to study CO_2 fixation by heterotrophic and autotrophic bacteria have shown that such exchange is very unlikely. In fact, if there was direct exchange, say, between $R - C^{12}OOH$ and $C^{14}O_2$, we would observe α-, and not γ-carboxylation of pyruvic acid according to the Wood-Werkman reaction. There is practically no incorporation of C^{14} into organic acids in the presence of purified carbcxylase. Such incorporation occurs only in the presence of an unpurified preparation, when oxidation, providing an energy source, occurs (Utter and Wood [1951]). In the autotrophic assimilation of CO_2 its carbon does not appear in the carboxyl groups of organic acids, but in phosphoglyceric acid, where it arrives by incorporation in ribulose diphosphate (Aubert et al. [1957]). When C. kluyveri is grown in the presence of labeled carbonate, according to Tomlinson and Barker [1954], C^{14} appears in the methyl group of acetic acid, and not in the carboxyl group. If an exchange took place the C^{14} would have appeared immediately in the carboxyl group. More such examples could be given.

Much more probable would be direct exchange between $C^{14}O_2$ and $HC^{12}OOH$ in cultures of bacteria oxidizing formic acid and possessing a corresponding enzyme complex. To demonstrate this exchange we distilled the formic acid residue at the end of the experiments, concentrated it by evaporation and analyzed it for the presence of C^{14}. These analyses did not reveal any incorporation of C^{14} from carbonates into formic acid (Table 1). Thus, there was no exchange of C^{14} between carbonate and formic acid in the bacterial culture. The likelihood of such an exchange in the experiments with the other, more stable, organic acids was even less.

The results of experiments to determine the degree of utilization of CO_2 in biosynthesis in our isolated organism, which we called Bacterium formoxidans sp. n., are given in Tables 1-3.

TABLE 1. Role of CO_2 Carbon in Biosynthesis in Bacterium formoxidans sp. n. Growing in a Medium Containing Na Formate.

Carbon compound	Carbon content of sample taken for analysis, mg	Radioactivity of carbon in sample taken for analysis, counts/mg C	Specific activity of carbon, counts/mg C	% of bacterial body carbon assimilated from CO_2 (with correction for isotopic effect)
CO_2+HCO_3 (start of expt)	4.9	34100	6950	95.5
CO_2+HCO_3 (end of expt)	1.92	12020	6240	-
Organic matter of bacteria	4.8	28600	5950	-
Formic acid at end of expt.	7.2	0	0	-

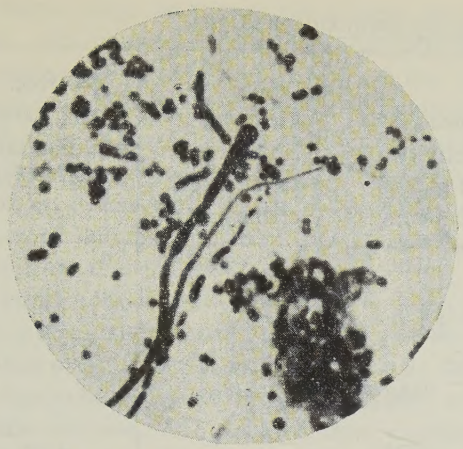


Fig. 2. Smear from culture on medium containing mannitol. 2000 ×.

Fig. 3. Smear from culture on medium containing sucrose. 2000 ×.

Table 1 shows the results of a determination of CO_2 carbon utilization for biosynthesis when Bacterium formoxidans sp. n. was cultured on a medium with nonradioactive Na formate and with a high concentration of labeled bicarbonate in the medium. In these experiments the dilution of the bicarbonate label by the evolution of CO_2 in the oxidation of nonactive formic acid by the bacterial cells was reduced to a minimum. When bacteria were grown in these conditions, as the given data show, the specific activity of the bacterial body carbon was close to the specific activity of the carbonate carbon. Determinations showed that in oxidizing formic acid the bacteria obtained more than 90% of their body from CO_2, i.e., they fed like chemosynthetic bacteria. In our approach to these experiments we had assumed that this organism used CO_2 carbon for biosynthesis on a greater scale than other heterotrophs, since it has to construct its body substance from a one-carbon compound. But the results which we obtained were totally unexpected. The conclusion that bacteria oxidizing formic acid were chemoautotrophic seemed rather paradoxical, but the experimental data were perfectly reliable. There was no possibility of exchange reactions between $C^{14}O_2$ and HCOOH since we did not find even traces of C^{14} in the formic acid carbon at the end of the experiment. Moreover, if such an exchange occurred, the bacterial bodies would have the specific activity of the carbonate only if they did not assimilate formic acid carbon, since the latter exchanged inside the cell with the carbon of labeled HCO_3 would dilute the HCO_3 label. In this case the specific activity of the bacterial bodies would not be 90% of the specific activity of HCO_3, but about 50%.

To verify the conclusion on the autotrophy of Bact. formoxidans we conducted experiments in which the cultures were grown on labeled formic acid in the presence of a high concentration of unlabeled carbonate. We subsequently analyzed the specific activity of C^{14} in formic acid, HCO_3-, and in the bacterial bodies. The results are given in Table 2. These data confirm the conclusion that Bacterium formoxidans sp. n. is autotrophic. In fact, the specific activity of C^{14} in the organic matter of the bacteria was not close to the specific activity of formic acid, but was almost equal to the mean specific activity of HCO_3 - during the experiment.

The radioactive carbon appeared in the HCO_3- of the medium as a result of the oxidation of formic acid to CO_2. There was no exchange between $HC^{14}OOH$ and CO_2 in this case either. This can be seen from the results of the analysis of the specific activity of formic acid, which was the same at the beginning and end of the experiment. If we assume that 97% of the C^{14} passed into the cells from HCO_3- carbon (Table 1), we can use the formula given above to calculate how much C^{14} was assimilated by bacteria from the formic acid. We found that this value was only about 1-4%.

In subsequent experiments with labeled formic acid we tried to minimize the incorporation of C^{14} into the substance of the bacterial bodies due to assimilation from the HCO_3- and CO_2 of the medium, which gain C^{14} from the breakdown of labeled formic acid by the bacteria. The bacteria were grown in a buffered medium containing labeled formic acid (10 g/liter) and a small amount of HCO_3-(0.1 g/liter). The medium was contained in a tall cylinder of about 3-liter capacity and a mixture of air and CO_2 (5:1) was blown through it. The CO_2 blown through the medium was exchanged with the CO_2 of the medium, and the flow "washed out" the molecules of labeled bicarbonate appearing in the medium as a result of the breakdown of labeled formic acid. During the experiment we determined the changes in the specific activity of the formic acid and HCO_3- of the medium. The results are given in Table 2. They show that during the experiment the specific activity of HCO_3- never rose above 230-400 counts/mg, which was only 10% of the specific activity of C^{14} in the formic acid. The specific activity of the formic acid did not change during

TABLE 2. Assimilation of Carbon of Labeled Formic Acid by Bacterium formoxidans sp. n.

Experimental conditions	Carbon content	Carbon content of sample taken for analysis, mg	Radioactivity of carbon in same sample, counts/mg C	Specific activity of carbon, counts/mg C	Mean specific activity during expt, counts/mg C	% carbon assimilated by bacteria from formic acid
High HCO_3- content; closed vessel	Formic acid (start of expt)	2.88	157000	55000	54900	-
	The same (end of expt)	4.02	220000	54800	-	-
	CO_2 and HCO_3 (end of expt)	6.5	49500	7650	3825	-
	Organic matter of bacteria (end of expt)	4.4	11000	4350	-	1.60
Low HCO_3- content; CO_2 + air mixture blown through medium	HCO_3+CO_2 (after 3 days)	4.6	2030	440	312	-
	The same (after 4 days)	7.05	1600	226	-	-
	The same (after 6 days)	6.5	1600	245	-	-
	Formic acid (start of expt)	5.94	30100	5080	5083	-
	The same (after 4 days)	6.56	33600	5120	-	-
	The same (after 6 days)	6.0	30400	5050	-	-
	Organic matter of bacteria (after 4 days)	3.17	1500	471	490	4.3
	The same (after 6 days)	1.56	725	510	-	-

TABLE 3. Assimilation of CO_2 Carbon by Bacterium formoxidans Growing in Defined Medium with Addition of Various Organic Substrates. Initial Specific Activity of C^{14} in CO_2 and HCO_3- of Medium = 12 080 counts/mg C

Substrate	Specific activity of C^{14} in CO_2 and HCO_3- at end of expt, counts/mg C	Mean specific activity of C^{14} in CO_2 and HCO_3-, counts/mg C	Specific activity of C^{14} in organic matter of bacteria, counts/mg C	% bacterial body carbon derived from CO_2
Formic acid	95200	10800	9957	97.7
Acetic acid	10950	11565	1141	10.55
Lactic acid	10800	11440	828	7.61
Glucose	10200	11060	418	3.82
Glucose + peptone	11100	11540	320	2.94

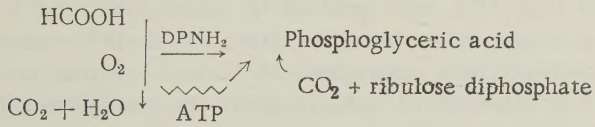
the experiment. Thus, there was no exchange between $HC^{14}OOH$ and $HC^{12}O_3$- in the growing culture even when one of the products of this reaction was constantly removed. Hence, the obtained data are perfectly reliable. They show that in these conditions formic acid C^{14} enters the bacterial bodies in insignificant quantity. The specific activity of the bacterial bodies in this case was a tenth of the specific activity of formic acid. This demonstrates the autotrophic nature of the nutrition of Bacterium formoxidans.

It is not quite clear why the bacteria do not assimilate the intracellular carbon dioxide formed in the oxida-

tion of formic acid, but take carbon dioxide from the medium. This is obviously due to the fact that the bacteria possess systems which accomplish, on one hand, active assimilation and, on the other, active excretion of metabolic end products. We can easily conceive that these systems can be spatially and functionally disconnected if we assume that they are accomplished on the surface of the cell membrane. The data given above indicate in favor of the latter.

In view of the fact that Bacterium formoxidans grows well on several other substrates, it was of interest to find out to what extent the consumption of CO_2 by this organism for biosynthesis depends on the complexity of the oxidizable substrate. The results of these experiments are given in Table 3, which shows that in growth on organic substrates more complex than formic acid the bacterial body substances are constructed, not from CO_2 carbon, but from the carbon of the oxidizable substrate. In this case carbon dioxide is only a subsidiary source of carbon. The degree of its participation in biosynthesis depends on the complexity of the oxidizable substrate. For instance, in the oxidation of acetic acid more CO_2 is used in biosynthesis than in the oxidation of glucose or lactic acid. In the presence of peptone, CO_2 assimilation becomes quite insignificant. The results of these experiments are perfectly consistent with the known facts of the reduction of the CO_2 requirement for growth of some bacteria in synthetic media when dicarboxylic acids and amino acids are added (Kupletskaya [1955]).

How are we to represent the metabolism of Bacterium formoxidans in autotrophic conditions? The general scheme of the metabolism of this bacteria will presumably be typical of all autotrophic bacteria:



According to this scheme, formic acid is oxidized to CO_2 and H_2O; the energy of oxidation is bound in high-energy phosphates (ATP type and polyphosphates) and reducers of the $DPNH_2$ type, which are used for CO_2 fixation and the construction of primary carbohydrates via the ribulose cycle, as in the case of photosynthesis. Primary fixation of CO_2 in all the chemosynthetic bacteria studied from this aspect is effected mainly via the ribulose cycle. This cycle obviously plays a part in CO_2 fixation in Bacterium formoxidans. This hypothesis is borne out by Quayle and Keech's results [1958], which showed that during oxidation of formic acid Pseudomonas oxalaceticus fixes CO_2 by the ribulose cycle.

The inability of Bacterium formoxidans to utilize formic acid carbon for biosynthesis is possibly due to the fact that the first stages of biosynthesis are effected via the ribulose cycle, in which CO_2, and not HCOOH, is the initial component.

DISCUSSION

According to published information, many bacteria oxidize formic acid in aerobic conditions (Kolesnikova [1959]). This ability has been noted in representatives of the Escherichia coli group (Omelyanskii [1953], Cook and Stephenson [1928]), Pseudomonas fluorescens, Proteus vulgaris, and several other saprophytic bacteria (Maasen [1896]). Omelyanskii isolated a formate-decomposing facultative anaerobic bacterium – Bacterium formicum. In many characters this bacterium resembles the coli group. Under anaerobic conditions Bacterium formicum ferments formic acid and produces hydrogen and carbon dioxide (Omelyanskii [1953], Kolesnikova [1957, 1959]).

All the above forms oxidized formic acid when they were grown in protein and defined media with the addition of peptone or amino acids, and did not grow in media where Na formate was the only source of carbon.

Of much greater interest are the reports of the isolation of cultures of aerobic bacteria using formic acid as the only source of carbon. Loew [1892] described a bacterium which grew in a 0.5% solution of sodium formate, and in solutions of methyl alcohol or formaldehyde.

According to Bhat's results [1956], some strains of P. fluorescens grow in defined media containing formates. It has been reported that Hiphomicrobium vulgare (Näveke and Engel [1955]) and Pseudomonas oxalaceticus (Quayle and Keech [1958]) have similar properties.

Our isolated facultative autotroph oxidizing formic acid differs from all previously described species of bacteria. This organism can be assigned to an independent species, which we call Bacterium formoxidans sp. n.

The diagnostic characters of this species are as follows: Shape of cells in young cultures – solitary short rods, sometimes almost coccoid (Fig. 1). As the cultures become older the cells become larger, and polar granularity becomes apparent. Cell dimensions: in young cultures – 0.6-0.8 × 0.8-1.1 μ, in old cultures – 1.1-1.3 × 1.4-2.5 μ. Cells nonmotile and nonflagellate. Gram-negative. Aerobic. Temperature optimum 30 C. Grows in mineral medium with addition of formate as sole source of carbon. When growing in this medium it metabolizes like an autotroph. Grows well in defined media containing acetate, lactate, ethyl alcohol, glucose, or lactose. Growth is weaker in media containing mannitol or sucrose and characteristic involution forms appear (Figs. 2, 3). Does not produce acids and gas from sugars. Does not use methanol or oxalic acid. Grows slowly in bouillon. Forms gas in bouillon containing KNO_3. Grows very weakly on gelatin and does not liquefy it. Grows well on meat-peptone agar. Streak on meat-peptone agar white, smooth, glistening, raised, of paste-like consistency. Grows very slowly on potato and produces a slimy streak tinged with brown. Growth very slow in medium containing starch and in milk. Milk is weakly alkalized.

In Krasil'nikov's key [1949] Bacterium formoxidans sp. n. belongs to the group of species in the genus Bacterium of "undetermined systematic position." In a number of characters it resembles Bacterium candicans.

SUMMARY

1. An aerobic bacterium growing in a defined medium with formate as the only source of carbon was isolated. The diagnostic characters of this microorganism, described as a new species, are given.

2. It was shown by means of C^{14} that Bacterium formoxidans sp. n. synthesizes its body almost entirely (95-97%) from carbon of CO_2 contained in the medium. The carbon of formic acid, like the carbon of CO_2 directly formed from the latter by oxidation, is not used for biosynthesis.

3. The evidence obtained suggests that the production of carbon dioxide by dissimilation and the assimilation of carbon dioxide in biosynthesis take place on the surface of the cell membrane in this organism and are spatially disconnected.

LITERATURE CITED

N. A. Krasil'nikov, 1949, Key to Bacteria and Actinomycetes [in Russian] (Izd AN SSSR).

I. G. Kolesnikova, 1957, Mikrobiologiya 26, 5.

I. G. Kolesnikova, 1959, Uspekhi sovremennoi biol. 47, 1.

M. B. Kupletskaya, 1955, Tr. In-ta mikrobiol. AN SSSR 4, 3.

V. L. Omelyanskii, 1953, Selected Works (Izd. AN SSSR).

Yu. I. Sorokin, 1960a, Doklady Akad. Nauk SSSR 132, 2.

Yu. I. Sorokin, 1960b, Mikrobiologiya 29, 2.

J. P. Aubert, I. Milhaurd, and I. Millet, 1957, Ann. Inst. Pasteur 92, No. 4.

I. Bhat, 1956, J. Indian Inst. Sci. 38, No. 1, 2.

R. P. Cook and M. Stephenson, 1928, Biochem. J. 22, 1368.

O. Loew, 1892, Ztbl. Bakt. 12, 462.

R. Näveke and H. Engel, 1955, Arch. Mikrobiol. 21, No. 4, 371.

A. Maasen, 1896, Arb. aus den Kaiserl Lesundheitsamte 12, 340.

I. Quayle and D. Keech, 1958, Acta Biochem. Biophys. 29, 233.

N. Tomlinson and H. A. Barker, 1954, J. Biol. Chem. 209, 585.

F. Utter and H. I. Wood, 1951, Adv. Enzymol. 12, 42.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

SYMBIOTIC CULTURE OF A NEW MICROORGANISM OXIDIZING MANGANESE

G. A. Zavarzin

Institute of Microbiology, Academy of Sciences, USSR
Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 393-395, May-June, 1961
Original article submitted February 1, 1961

A recently-discovered unusual microorganism (Frantsev, 1959) plays an important part in the precipitation of manganese in bodies of fresh water.

When a sediment of iron bacteria from a muddy brook was plated on Liske medium with manganese acetate, colonies of a fungus which deposits manganese in the form of regular concentric rings grew on the plates (Fig. 1). Thorough microscopic examination revealed that all of the oxidized manganese was located between the fungal hyphae, which were free of oxides, on the surface of brown spherical microcolonies consisting of radially diverging, coiled filaments. By isolating single colonies and transferring them successively on the same medium, a culture was soon obtained containing only the manganese-oxidizing organism and the fungus.

All attempts at further separation proved to be unsuccessful. When separated from the fungus by filtration, the manganese-oxidizing organism failed to grow on any of the media tested. At the same time, all hyphal fragments of the fungus contained germs of this organism. It was possible to detect the independent multiplication of the manganese-oxidizing organism by counting, in a Goryaev chamber, the microcolonies in a culture from which the mycelium of the fungus had been removed by filtration through a glass filter. Subcultures from the filtrate gave no growth(see table). The addition of corrosive sublimate to the filtrate halted multiplication completely.

On the other hand, by changing the conditions of the medium, the predominant development of either the fungus or the manganese-oxidizing microorganism could be achieved.

The symbiotic culture grows in a very simple medium. The following components are required: 1) polysacchraides, 2) manganese, 3) water.

1) Polysaccharides. Growth occurred on agar, gum arabic, starch, glycogen, cellulose, and inulin. The best results were obtained with 0.1-1.5% agar, 2% gum arabic, and 2% starch. Growth depends on the concentration of these substances; it does not occur in solutions that are too dilute. Monosaccharides, especially glucose, inhibit oxidation even at low concentrations.

2) Manganese. The organism has poor tolerance for high concentrations of dissolved manganese. The best growth was on the slightly-soluble manganese salts: manganese carbonate and oxalate. Manganese acetate began to inhibit oxidation at concentrations above 0.02%.

3) Water. Growth was found only in media prepared with distilled water; furthermore, traces of copper in the water favored growth. The addition of diethy dithiocarbamate and thiourea, which bind copper, stopped oxidation. The addition of 0.1 μM of copper sulfate to purified medium stimulated growth. The requirement for other elements was so small that it was not detected under ordinary experimental conditions.

The pH values favorable for the growth of the fungus and for manganese oxidation are different. Manganese oxidation ceases at pH 5.5, although the fungus grows at this pH. On the other hand, when the pH is raised, the rate of manganese oxidation increases, the most favorable pH values being 6.8-7.2.

Content of Microorganisms in Thousands of Microcolonies per 1 ml

Initial	First day	Second day	Remarks
199	602	857	
350	659	–	Average of seven parallel experiments
52	–	144	The same

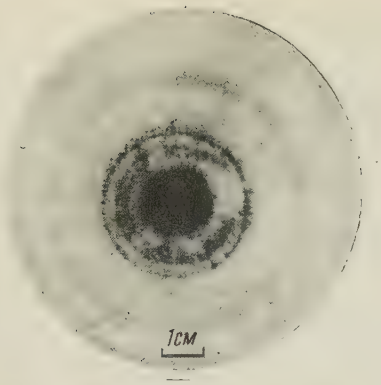


Fig. 1. Joint colony of M. symbioticum and fungus on medium with manganese acetate. The sites of growth of M. symbioticum are a dark color due to manganese oxides and are arranged in concentric rings.

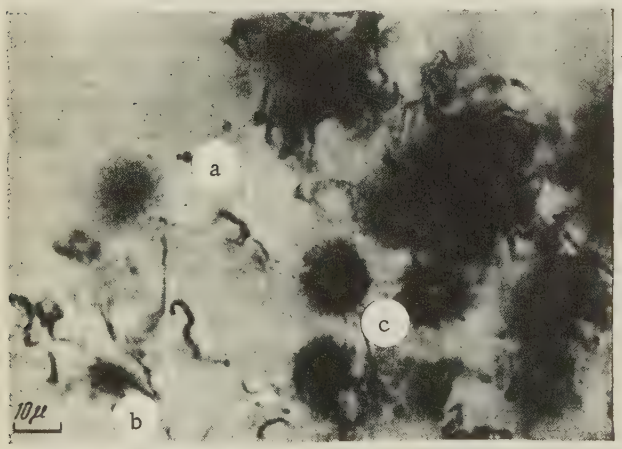


Fig. 2. Growth of M. symbioticum on medium containing starch and manganese carbonate. A large number of free motile buds (a) can be seen, as well as free filaments (b) and microcolonies (c) covered with manganese oxides.

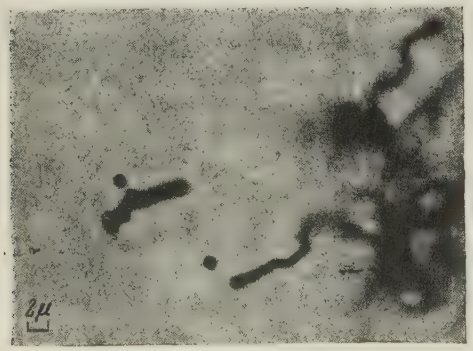


Fig. 3. Formation of a motile bud on a filament fragment. It was further possible to observe how the bud detached and actively swam away.

Therefore, the following media can be recommended for a symbiotic culture of the manganese-oxidizing organism and the fungus:

I. Liske medium. Leached agar – 1.5%, manganese acetate – 0.01%.

II. Semisolid medium. Leached agar – 0.1%, manganese oxalate or carbonate – one drop of thick suspension per tube containing 5 ml of medium.

III. Liquid medium. Starch – 2%, manganese carbonate – 0.1%. Water is distilled in all cases.

The joint colonies of the fungus and manganese-oxidizing organism on solid media have a very characteristic concentric structure, with the fungal hyphae always outstripping the growth zones of the manganese-oxidizing organism. The fungus forms no fruiting bodies in the zones of manganese oxidation, and the identification of the fungus is therefore difficult.

The manganese-oxidizing organism isolated in symbiotic culture is known to have a complex growth cycle. The most characteristic and easily distinguished stage is the spherical microcolony measuring several tens of microns in diameter (Fig. 2). In the center of the colony is a large spot, which is completely filled with manganese precipitates. Irregularly coiled filaments, tapering toward the periphery, radiate laterally from this spot. The diameter of the filament at the base varies depending on the age of the colony. The outside end is less than 0.1μ and is beyond the limits of the resolving power of the microscope. This "little spider" stage remains in the culture for quite a long time, but the spaces between the filaments are eventually filled in with manganese oxides, and the microcolony becomes a formless little lump in which it is impossible to recognize any structural elements under the microscope.

In the starch medium, at a certain point in the development of the culture, round buds measuring about 1μ in diameter and sitting on short stalks appear on the filaments. The buds actively separate from the filament and swim away (Fig. 3). Apparently, these buds further germinate as coiled filaments.

As far as can be determined by observations in the anopteral and electron microscopes after either dissolving the manganese oxides with 0.1 N oxalic acid or binding them, old filaments contain no cellular formations inside the filament (Figs. 4-6).

DISCUSSION

The absence of cells inside the filament does not permit one to relate the isolated organism to Leptothrix echinata Beger (1935), which it resembles by the shape of the microcolonies and ability to oxidize manganese. At the same time, it greatly resembles an organism described by Guseva (1956), differing from it noticeably in shape of filaments. A comparison of the isolated organism with plankton preparations kindly placed at our disposal by Guseva compels us to regard the isolated organ-

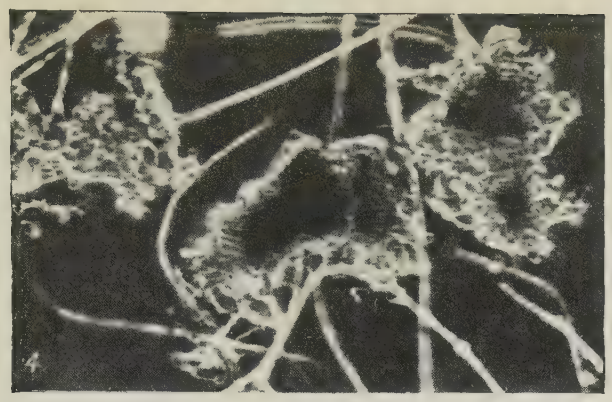


Fig. 4. An old, highly mineralized microcolony of M. symbioticum, closely bound to fungal hyphae. The next photographs show successive stages in the dissolution of manganese oxides by 0.1 N oxalic acid. Anopteral microscope.

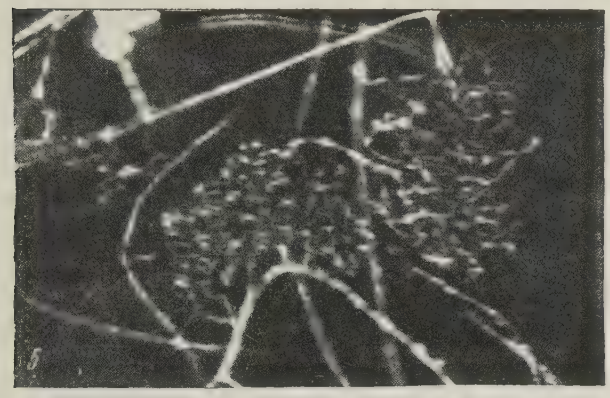


Fig. 5. The same microcolony, but partly dissolved by oxalic acid. The filamentous structure stands out clearly.

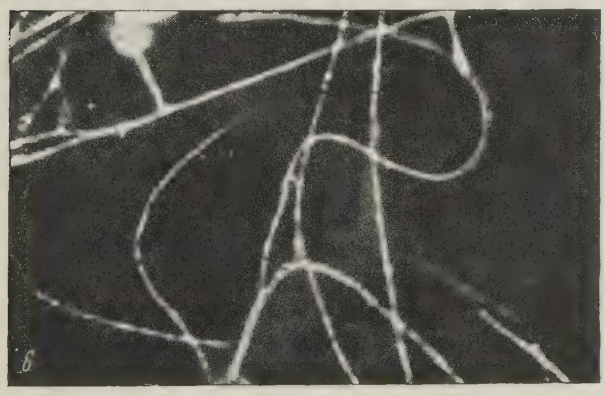


Fig. 6. The microcolony after complete elimination of manganese oxides. Only fungal hyphae and small bubbles of carbon dioxide gas formed in the process of dissolving the oxides are seen.

ism as a similar, but independent, species. In describing an analogous organism, Perfil'ev (1959) proposed the name Metallogenium personatum, pointing out that the microcolonies consist of small coccoidal budding cells connected by extremely fine filaments, and furthermore, "that its isolation into pure culture by standard methods is impossible" (p. 37). Although we do not share this opinion, it nevertheless seems possible to place the isolated organism in the genus Metallogenium Perf., naming it Metallogenium symbioticum sp. n. The objection that can be made against the separation of the isolated organism into a new species is that M. personatum and M. symbioticum were observed under different conditions: the first – only in a natural environment, and the second – in culture. However, the absence of cells in the filaments is a sufficiently important characteristic on which to base the formation of a new species.

The growth cycle of the organism and its structure generally resemble Hyphomicrobium vulgare (Zavarzin, 1960). Reproduction by budding and the presence of filamentous appendages indicate its relationship to the order Hyphomicrobiales Douglas.

SUMMARY

1. A new manganese-oxidizing microorganism has been detected and isolated in a symbiotic culture.

2. The organism grows in a simple defined medium containing only a polysaccharide and salts of divalent manganese, but requires the presence of a fungus for its development.

3. The organism, which has been named Metallogenium symbioticum sp. n., belongs to Hyphomicrobiales.

LITERATURE CITED

1. K. A. Guseva. 1956. Tr. biol. stantsii "Borok" 2: 24.
2. G. A. Zavarzin. 1960. Mikrobiologiya 29: 38.
3. B. V. Perfil'ev. 1959. The Theory and Technic of Continuous-Flow Bacterial Microcultures. Stenogram of report [in Russian] (Izd. LGU, 1959).
4. A. V. Frantsev, Trudy Vsesoyuz. Gidrobiol. Obshchestva 9, 13 (1959).
5. H. Beger. 1935. Ztbl. Bakt., Abt. II, 92: 401.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

A CONTRIBUTION TO THE SPECIES COMPOSITION OF ACTINOMYCES OF CERTAIN DISTRICTS OF SOUTHERN CHINA

T. S. Maksimova, T. P. Preobrazhenskaya,
E. S. Kudrina, and M. A. Sveshnikova

Institute for New Antibiotics Research, Academy of Medical Sciences, USSR
Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 396-401, May-June, 1961
Original article submitted May 24, 1960

Among soil microorganisms considerable attention has been given to Actinomyces as producers of antibiotics. In an investigation of new antibiotics a test of practical application indicates that, in the majority of cases, different species of microorganisms form different antibiotics, although the facts of production of one or another antibiotic for different species are well known. From this point of view both an investigation of the species composition of microorganisms (Actinomyces in particular) and the degree of their distribution in various geographical regions offers considerable practical and theoretical interest.

Actinomyces are found in the soils of various geographical areas. Investigation of the species composition of Actinomyces of certain regions has been carried out by a number of workers. Within the boundaries of the USSR, for example, Actinomyces composition of the soil of various areas was investigated by Krasil'nikov, Korenyako, Artamonova [1953], Kriss [1947], Solov'eva and Taig [1959], Egorov and Polin [1955] and others.

An investigation of Actinomyces antagonists has also been carried out in other countries. In Bulgaria these questions were studied by B'rdarov and Gushcherov [1956], in Poland – Golebiowska and Wrober [1956], in Korea – Doki Chun [1956], and so forth.

In the Institute for New Antibiotics Research an investigation of Actinomyces isolated from 1,580 soil samples from five of the provinces of Southern China was conducted during the years 1956-60. The aim of the current work was an investigation of the species designation of isolated Actinomyces in relation to soil types and the determination of the dominant forms among the antagonists and non-antagonists.

The investigation included samples of red and yellow soils – 795 samples, and alluvial soils – 611 samples (here we took gley and oryzeae soils). Futhermore a small number of samples of deluvial limestone, lake loam, and other soils were investigated.

METHODS

Soil samples were sifted twice in Petri dishes with six different media: potato, Gauze's mineral and organic media, pea, Emerson medium, and Umedzav medium. After incubation for 5-6 days at 28 C protuberances of

TABLE 1. Number of Actinomyces Antagonists in Various Soil Samples.

Province	Soils	No. of Samples Tested	No. of Actinomyces Tested	Antagonists to Tests		Non-Antagonists to Tests	
				No. of Strains	%	No. of Strains	%
Kwangsi	Red and Yellow Soils; Alluvial	401	2381	1440	62	941	38
Kwangtung	The same	337	1652	825	50	827	50
Kiangsi	Alluvial	195	908	520	57	388	43
Hunan	Red and Yellow Soils	201	1605	951	59	654	41
Szechwan	Alluvial, Gley, Red Yellow Soils	346	1570	887	57	683	43
	Total	1480	8116	4623	57	3493	43

TABLE 2. Dominant Species of Antagonists and Non-Antagonists in Various Soil Types

Soils	Dominant species of antagonists: species	total no. of strains	% of antagonists	Dominant species of non-antagonists: species	total no. of strains	% of antagonists
Red and yellow	Act. griseus v. purpurescens	332	94	Act. olivaceus	143	85
	Act. globisporus	907	77	Act. griseovariabilis	394	52
	Act. griseus	682	72	Ast. griseus	682	28
	Act. griseovariabilis	394	48			
Alluvial	Act. griseus v. purpurescens	262	91	Act. olivaceus	222	92
	Act. chromogenes	98	65	Act. coeruleofuscus	55	90
	Act. griseus	712	63	Act. griseovariabilis	240	69
	Act. griseovariabilis	240	31	Act. griseus	712	37

Actinomyces were sealed in slants of agar mineral medium No. 1. *

The antagonistic properties of Actinomyces were tested by using streaks on organic medium No. 2.* The test microbes used were Staphylococcus aureus, Escherichia coli, Bacillus mycoides, and Candida albicans.

In establishing the systematic position of isolated Actinomyces the identifications of Krasil'nikov [1949], Waksman [1953], Gauze and others [1957] and the descriptions of species in periodical publications were used.

From the soil samples received 8,116 cultures were isolated – 106 strains from various sources failed to develop their systematic position.

Determination of the systematic position of the isolated cultures was made on the basis of the morphological and cultural characteristics of the Actinomyces. Strains similar in morphological and cultural characteristics but differing in antibiotic spectrum were considered by us as a single species, inasmuch as we considered antimicrobe spectrum and physiological characteristics as complementary and not as basic indications in determination of species.

With the knowledge of the species composition of Actinomyces we had to take into account, as further investigation with the electron microscope indicated, that Actinomyces griseus is a heterogenous species and has three types of sporecase [Preobrazhenskaya and others, 1960]. Strains with smooth spores were identified as Actinomyces albogriseus, strains with echinate spores were identified as Actinomyces noursei, and strains with hairy spores were left as Actinomyces griseus.

RESULTS

Data from the investigation of soil samples are shown in Table 1.

As the references [Waksman, 1942; Bergolder, 1946; Krasil'nikov and others, 1950, 1953, and others] in the literature concerning the number of Actinomyces antagonists indicate the percentage of antagonists varies from 5% in peat to 40-70% in the forest soils of the Black Sea coast [Krasil'nikov and others, 1953]. According to Egorov and Polin [1955] there are approximately 76% antagonists in the soils from the river Lena's middle course.

Analysis of the antibiotic characteristics of the Actinomyces of Southern China showed that 57% of the cultures had antimicrobial properties (Table 1). Among the cultures of antagonists and non-antagonists isolated from various types of soil, predominant species were found. These data are shown in Table 2.

Predominant species among the antagonists were Actinomyces griseus var. purpurescens – 81-94%, A. globisporus – 77% and A. chromogenes – 65%. A. olivaceus and A. coeruleofuscus were shown in the majority of cases not to possess antimicrobial characteristics. Thus the percentage of non-antagonists for A. olivaceus comprised 85-92%, for A. coeruleofuscus – 90% of the total number of strains of the species. Among the widespread species A. griseus and A. griseovariabilis are encountered some antagonists and some non-antagonists.

The investigation of the systematic position of isolated strains indicates a large number of species of Actinomyces in Southern China. We isolated 98 species, representing all the series of Actinomyces. Species with pale yellow aerial mycelium (series helvolus) and with gray aerial mycelium (series griseus, aureus and violaceus) predominated.

Actinomyces with rose and blue aerial mycelium were encountered most often in the soil types studied.

Many authors have noted a predominance in various soil types of Actinomyces with gray, pale yellow, and white aerial mycelia. Thus, Rybalkin and Kononenko [1959], investigating the species composition of Actinomyces in tundra, podzolic and chernozem soils, pointed out the widespread distribution in specific soil types of

* For the composition of media No. 1 and No. 2 consult Gauze's monograph [1957].

TABLE 3. Distribution of Dominant Forms of Actinomyces in Various Soil Types of Southern China.

Series	Most widely distributed species	No. of strains in various soils			
		alluvial and oryzeae	red and yellow	deluvial lime-stone	lake loam
Lavendulae-roseus	Act. lavendulae	92	70	4	—
		36	42	6	—
	Act. roseolus	11	7	—	—
	Act. toxytricini	7	1	—	—
	Act. venezuelae				
Fradiae	Act. roseofulvus	18	15	—	—
Fuscus	Act. cinnamonensis var. proteoliticus	13	27	—	—
	Act. daghestanicus	1	14	—	—
Albus	Act. longisporus	13	38	—	—
Helvolus	Act. globisporus	70	97	4	—
	Act. globisporus v. flavofuscus	35	50	12	—
	Act. streptomicini	15	13	—	—
	Act. rubiginosohelvolus	18	20	—	3
	Act. griseus v. purpurescens	262	332	5	10
	Act. globisporus v. caucasicus	13	27	—	—
	Act. hachijoensis v. fuscus	10	2	—	—
Griseus	Act. griseus	712	682	—	3
	Act. griseovariabilis	240	394	11	3
	Act. diastaticus	44	82	2	3
	Act. rubiginosus	122	102	—	—
	Act. rochei	40	82	—	—
	Act. flavoviridis	47	121	—	—
	Act. flavochromogenes	67	39	—	—
	Act. flaveolus v. rectus	37	24	—	3
	Act. antibioticus	50	35	—	—
	Act. olivaceus	222	143	9	2
	Act. higroscopicus	29	17	4	—
Chromogenes	Act. chromogenes	98	83	8	7
	Act. variabilis	34	22	—	—
	Act. variabilis v. roseolus	39	38	—	—
Chrysomallus	Act. alboviridis	11	11	—	1
	Act. olivaceoviridis	24	19	—	—
	Act. malachiticus	5	6	—	—
Nigrescens	Act. nigrescens	36	52	5	—
Violaceus	Act. violaceus	24	21	—	—
	Act. violaceochromogenes	22	34	—	—
	Act. violaceoniger	9	3	—	—
	Act. violaceoniger v. crystal-lomycini	86	132	4	—
Violaceus	Act. griseoruber	21	26	—	—
	Act. griseorubiginosus	43	87	—	—
	Act. coelicolor	1	19	—	—
	Act. coelicolor v. achrous	4	12	—	—
	Ast. violaceorectus	28	25	3	—
Coerulescens	Act. coerulescens	28	22	—	—
	Act. coeruleofuscus	55	43	6	—
	Act. chartreusis	4	12	—	—

representatives of such species as A. globisporus, A. globosus, A. cylindrosporus, and A. viridis. Rather should this be A. coelicolor, A. fradiae and A. longisporus-ruber, for cultures of these species seem specific for chernozem soils. Egorov and Polin [1955] discovered six species of Actinomyces in an an investigation of soil samples, of which A. griseus and A. globisporus comprised more than 90% of all cultures. In the high altitude soils of the Pamirs, according to the data of Solov'eva and Taig [1959], a large percentage of cultures of A. globisporus and A. griseus is reported and rare cases of blue-violet Actinomyces. Resources were not available for issuing a full list of species detected by us, but we show in Table 3 information concerning the dominant species of Actinomyces isolated by us from various soils of China.

As can be seen from Table 3, among the Actinomyces those with white and pale yellow aerial mycelia predominate, especially the series helvolus. Thus, for example, a large number of strains represents A. griseus var. purpurescens, comprising 7.0% of the total number of cultures examined. Of widespread distribution in the soils examined were the species A. globisporus and A. globisporus var. flavofuscus.

Representatives of various series of Actinomyces with gray aerial mycelia have unequal distribution in the soils of the southern provinces of China. Among the series Chrysomallus and Chromogenes we observed a small number of dominant species – at that time as the series griseus, aureus and violaceus have a greater number of predominant species – representative of a significant number of strains.

Such species of the series griseus as A. griseus, A. griseovariabilis and A. rubiginosus comprised respectively 17.8 and 2.5% of the total number of Actinomyces examined. Among the species of the series aureus, A. olivaceus was recorded as predominant, having 4.5% of the total number of cultures; many also were representative of A. flavoviridis.

The soil investigation showed a large number of representatives of the series violaceus, among which were many dominant species.

As has already been mentioned, the Actinomyces with rose and azure aerial mycelia had a limited distribution in examination of the soils. First in the basic species representatives were A. roseolus, A. roseofulvus and A. cinnamonensis var. proteolyticus, and in the minority were strains of species A. toxytricini, A. daghestanicus and A. venezuelae.

Among the Actinomyces with azure aerial mycelia the most frequently found were A. coerulescens, A. coeruleofuscus and A. chartreusis.

Through an examination of the predominating forms in various soil types (Table 3) it may be seen that A. griseus, A. lavendulae, A. globisporus, A. rubiginosus, A. violaceoniger and A. chromogenes are found both in alluvial soils and in red and yellow soils approximately in equal numbers. Such widespread species as A. griseus var. purpurescens and A. griseovariabilis were more widely distributed in the red and yellow soils, but A. olivaceus – in alluvial soils. The species A. diastaticus, A. rochei, A. flavoviridis, A. griseorubiginosus and A. longisporus, found in conspicuous numbers, were overwhelmingly found in red and yellow soils, but A. flavochromogenes – in alluvial soils. A. daghestanicus is found for the most part in red and yellow soils.

With regard to deluvial limestone and lake loams, a very insignificant number of Actinomyces were isolated from them, and no conclusions were reached regarding them.

SUMMARY

1. An investigation of the systematic position and antibiotic characteristics of about 8 thousand Actinomyces isolated from the soils of Southern China was made.

2. Approximately 50% of the cultures had antagonistic characteristics in the tests. Establishment of the relationship between the number of antagonists and the species composition of Actinomyces was made.

3. A large species range of Actinomyces in the soils of Southern China (98 species) was made.

4. The most widespread species are Actinomyces griseus, Actinomyces globisporus, Actinomyces rubiginosus, Actinomyces chromogenes, Actinomyces violaceoniger, found both in alluvial soils and in red and yellow soils. Actinomyces griseus var. purpurescens, Actinomyces griseovariabilis, Actinomyces diastaticus, Actinomyces rochei, Actinomyces flavoviridis, Actinomyces griseorubiginosus, Actinomyces longisporus and Actinomyces daghestanicus are more widely represented in red and yellow soils than in alluvial soils. The species Actinomyces olivaceus and Actinomyces flavochromogenes are distributed mainly in alluvial soils.

LITERATURE CITED

G. F. Gauze and others, 1957. Questions on the Classification of Actinomyces antagonists [in Russian] (Medical Press, Moscow).

N. S. Egorov and A. N. Polin, 1955. Mikrobiologiya 24, 1.

N. A. Krasil'nikov, 1949. The Identification of Bacteria and Actinomyces [in Russian] (Acad. Sci. USSR Press, Moscow).

N. A. Krasil'nikov, 1950. Actinomyces-antagonists and Antibiotic Agents [in Russian] (Acad. Sci. USSR Press, Moscow).

N. A. Krasil'nikov, A. I. Korenyako and O. I. Artamova, 1953. Mikrobiologiya 22, 1.

A. E. Kriss, 1947. Mikrobiologiya 16, 5.

T. P. Preobrazhenskaya and others, 1960. Mikrobiologiya 29, 1.

A. V. Rybalkin and E. V. Kononenko, 1959. The Microflora of the European Part of the USSR [in Russian] (Moscow).

N. K. Solov'eva and M. M. Taig, 1959. Izvest. Akad. Nauk SSSR, ser. biol., 2.

S. B"rdarov and G. Gushcherov, 1956. Izvest. Botan. In-ta B'lgarsk, AN, 5.

P. R. Bergolder, 1946. J. Bact. 52, 503.

Doki Chun, 1956, Antibiot. and Chemotherapy 6, 5, 324.

I. Golebiowska and T. Wrober, 1956. Acta Microbiol. Polon. 5, 1-2.

S. A. Waksman, 1942. Soil Sci. 54, 281.

S. A. Waksman and H. Lechevalier, 1953. Guide to the Classification and Identification of the Actinomyces and Their Antibiotics (Baltimore).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

MORPHOLOGICAL PECULIARITIES OF THE ACTINOMYCETES Actinomyces lavendulae AND Actinomyces aureofaciens UNDER PROLONGED CULTIVATION IN A CONTINUOUS CULTURE

M. N. Bekhtereva and I. G. Kolesnikova

Institute of Microbiology, Academy of Sciences, USSR
Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 402-408, May-June, 1961
Original article submitted November 21, 1960

Bacteria, yeasts, and fungi multiply intensively in continuous culture, and when certain conditions are observed the majority of cells remain for prolonged periods in a condition corresponding to the logarithmic growth phase of the static culture.

Scientists have noted, however, that along with the young cells, cells of varying ages were present in the culture. Thus, for example, cultures of Bacillus subtilis and Bacillus pumilis grown by the three-step scheme (Malek et al, 1956) exhibited sporulating cells even in the first step – these occurred among young cells.

A cell variation was observed, likewise, in experiments with continuous cultivation of Propionibacter shermanii (Neronova and Ierusalimskii, 1959).

For obtaining acetone biologically, the industry employs the semi-continuous (battery) method. The acetone-butyl bacteria flowing out with the first fermentation fluid are usually rods of uniform length, motile, and unautolyzed– these are the characteristics of a young culture. We noted, however (Logotkin, 1958), that among the usual short bacterial cells there occur elongated threads or weakly motile rods, whose different morphological condition would also indicate their differing physiological state.

The structure of the ray fungi-actinomycetes and their developmental cycle are quite complex. Hyphae may start their development from spores, hyphal fragments, spherical plasm accumulations, torn hyphal sections. The intracellular structure changes during autolysis, the protoplasm breaks up into little grains, the cell wall thins out and dissolves. We observe a complete or partial autolysis of the whole thread. The cellular elements formed during autolysis may serve as the basis for development of new hyphae. Krasil'nikov (1958) has carried out detailed observations of the above.

During submerged cultivation, the stirring method, aeration, pH of the medium and other physico-chemical factors exert a great influence on the culture's condition. The form and size of the submerged mycelial hyphal aggregates, the length and thickness of the hyphae, as well as their antibiotic-synthesis ability, all depend on the above listed factors. A number of scientific papers bear this out (Pirt et al., 1959; Neronova, 1959; Bekhtereva et al, 1959).

Morphological changes in actinomycetes grown in a continuous medium have not previously been investigated, and to this we devoted the present work.

We previously began a study of continuous actinomycete cultures (Bekhtereva, Kolesnikova, 1959). The present work was aimed at studying, somewhat in detail, morphological mycelial changes occurring when actinomycetes are grown in a continuous culture medium. We studied cultures of Actinomyces lavendulae and Actinomyces aureofaciens. We employed the continuous culture method described earlier.

We used 500 ml glass vessels, with working capacities of 200 ml. We grew the cultures and obtained antibiotics by the one-step scheme. We also experimented with the two-step scheme, using vessels of identical volume (200 ml amounts).

Every day (and sometimes twice daily) we examined the culture's condition and antibiotic activity (by the dilution method, using Staphylococcus aureus strain No. 209 as the test organism), the pH, and other biochemical properties of the out-flowing culture fluid. The mycelia were fixed with Carnoy's mixture and the slides were stained with methylene blue.

The cultivation medium for A. lavendulae contained: glucose, soluble starch, ammonium sulfate, NaCl, corn extract. The medium entering the vessel was brought to pH 7.3 with NaOH. The time for continuous-culture growth in medium was from 7 to 38 days. Below we present the research data characterizing the mycelial morphology of A. lavendulae in continuous cultivation. In the first three cases growth of the culture was accompanied by intensive antibiotic formation. The morpho-

logic picture in these experiments, however, varied somewhat.

In one of the experiments growth took place without any antibiotic formation in the vessel. The actinomycete culture was grown in a medium of low pH (5.5) for 38 days.

In all the experiments, as a rule, when the stream of medium was first introduced into the vessel the cul-

TABLE 1. Changes in Antibiotic Activity of the Biomass and in the pH of the Medium upon Continuous Cultivation of A. lavendulae for 32 Days (average rate of medium flow – 16 ml/hr)

Indicators	Culture fluid before flow initiation	Duration of process, days									
		1–3	4–6	7–9	10–12	13–15	16–17	21–23	24–26	27–29	30–32
Activity in conditional units	$0.025 \cdot 10^6$	$3.4 \cdot 10^7$	$3.5 \cdot 10^{10}$	$1.3 \cdot 10^{10}$	$1.1 \cdot 10^{12}$	$3.3 \cdot 10^{12}$	$3.3 \cdot 10^{12}$	$1.0 \cdot 10^{12}$	$1400 \cdot 10^{12}$	$550 \cdot 10^{12}$	$300 \cdot 10^{12}$
Culture fluid pH	6.5	6.8	6.8	6.7	6.7	6.8	6.6	5.74	5.27	5.19	5.14
Biomass, g/100 ml	0.43	0.38	0.18	0.17	0.27	0.44	0.44	0.39	0.24	0.15	0.18

TABLE 2. Changes in Antibiotic Activity of the Biomass and in pH at Varying Flow Rates of Fresh Medium

Indicators	Culture fluid before flow initiation	Duration of process, days								
		average flow rate of fresh medium through culture vessel – 17.5 ml/hr				average flow rate of fresh medium through culture vessel – 50 ml/hr				
		1	2	3	4	6	7	8	9	10
Activity in conditional units	$0.8 \cdot 10^8$	$5.0 \cdot 10^5$	$2.5 \cdot 10^5$	$20 \cdot 10^6$	$1 \cdot 10^4$	$5 \cdot 10^6$	$4 \cdot 10^4$	$20 \cdot 10^6$	$19 \cdot 10^9$	$20 \cdot 10^9$
Culture fluid pH	6.5	6.6	6.8	6.8	6.8	6.6	6.8	6.8	6.8	6.8
Biomass, g/100 ml	0.46	0.18	0.40	0.30	0.21	0.17	0.57	0.53	0.42	0.35

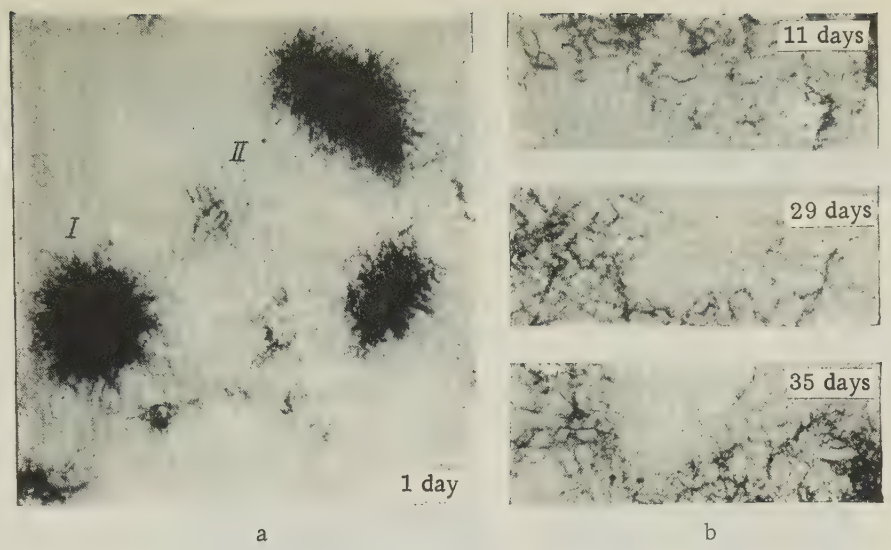


Fig. 1. Changes in mycelial aggregate types observed upon cultivation of A. lavendulae in a continuous medium. 800×. a) Dense hyphal aggregates (I), and appearance of slightly interwoven mycelial net (II) (first day of flow); b) spreading network of hyphae (on the 11th, 29th and 35th day of flow).

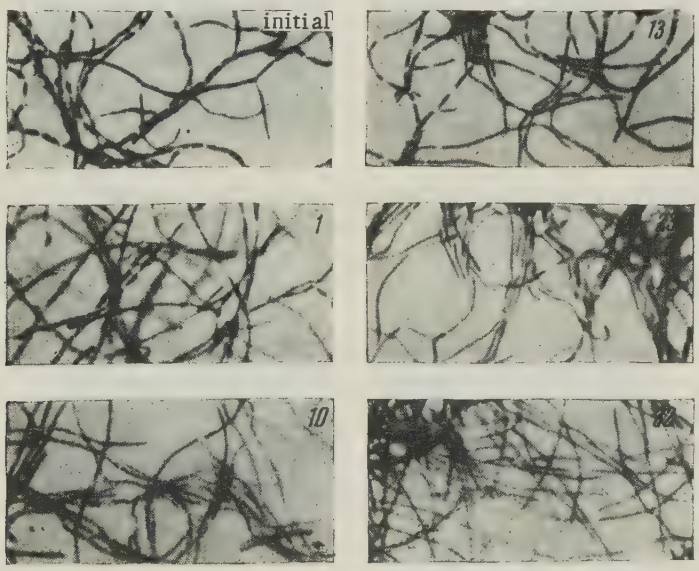
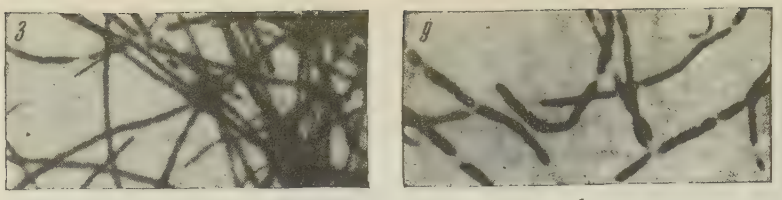


Fig. 2. Changes in A. lavendulae morphology upon cultivation in a continuous medium for 32 days (with intensive antibiotic production). 1600×. The majority of hyphae with strongly basophilic homogeneous or weakly differentiated protoplasm retain these properties throughout the streaming period. Numbers in upper right hand corner indicate age of culture in days.

ture abounded in hyphal aggregates with dense centers, branching into mycelial threads. As the fresh medium flowed in, the hyphal aggregate type was changed (Fig. 1, a, b). Among the densely matted aggregates there appeared again bunches of slightly interwoven threads. In the course of prolonged cultivation, their number increased until they filled the entire visual field, thus replacing the spherical aggregates.

First Series of Experiments. A. lavendulae was grown in the culture vessel for three days prior to introducing the fresh medium. The average speed of this stream was 16 ml/hour. Based on the formula Q=V/F, where F was the stream speed per unit of time, and V was the culture fluid volume in ml, the streaming time of medium through the culture vessel was 12.5 hr. The dilution coefficient D=0.085. The flow of medium was con-



a b

Fig. 3. Morphological changes in cultures of A. lavendulae producing antibiotic at various flow rates of medium. a) Speed of flow – 17.5 ml/hr, cultivation period 5 days, majority of hyphae had a homogenous, strongly basophilic protoplasm; b) speed of medium flow – 50 ml/hr, cultivation period 4 days; the main hyphal mass possessed a strongly basophilic, segmented, granular protoplasm, with a partial vacuolation.

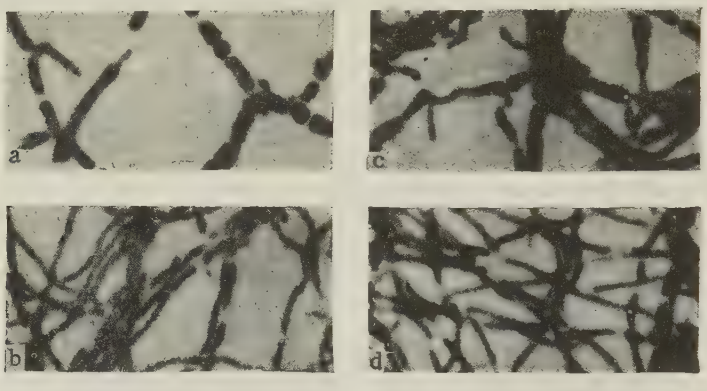


Fig. 4. Morphological characteristics of a mycelium encountered in continuous cultivation of A. lavendulae (with antibiotic production). 2400 x. a) Original culture; b) first day of growth; c) fourth day of growth; d) eighth day of growth.

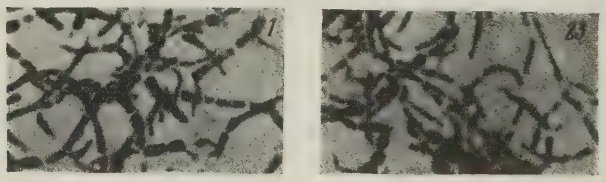


Fig. 5. Varied hyphae in A. lavendulae cultivated in a continuous medium. 1600 x (one and 23 days of growth).

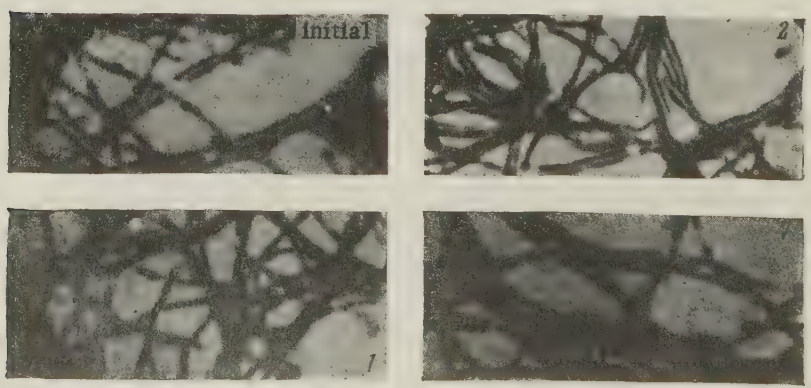


Fig. 6. Morphological changes in an A. aureofaciens culture grown in a continuous medium. Enlarged 2400 x (initial, 1, 2, and 7 days old).

TABLE 3. Changes in Biochemical Indicators of the Medium during Continuous Fermentation, Employing a Five-Day-Old Original A. lavendulae Culture (average speed of fresh medium flow through culture vessel – 25 ml/hr)

Indicators	Culture fluid before flow initiation	Duration of process, days							
		1	2	3	4	6	7	8	9
Activity in conditional units	$8 \cdot 10^5$	$8 \cdot 10^6$	$16 \cdot 10^6$	$1 \cdot 10^5$	$8 \cdot 10^8$	$3 \cdot 10^9$	$3 \cdot 10^9$	$41 \cdot 10^{10}$	$53 \cdot 10^{12}$
Culture fluid pH	6.0	6.2	6.0	6.1	6.0	6.8	6.0	6.3	6.3
Biomass, g/100 ml	0.50	0.34	0.46	0.3	0.33	0.39	0.25	0.36	0.23

tinued for 32 days. The culture produced antibiotic intensively during the whole period, and by the end of this continuous process the antibiotic yield was considerably increased (Table 1).

The morphological characteristics of the cultures are presented in Fig. 2. The main hyphal mass remained strongly basophilic during the 32 days, possessing a uniform protoplasm with some rare gaps. As the fresh medium streamed through, the culture was considerably rejuvenated as compared to its state before the initiation of the flow. However, concomitantly with the hyphae described above, we observed other mycelial threads. We encountered hyphae with a strongly vacuolated or a strongly basophilic, beaded protoplasm.

Second Series of Experiments. In these experiments we investigated how the flow rate of the medium through the culture vessel affects mycelial morphology and internal structure. The cultivation period was 10 days. During the first six days the rate of flow was 17.5 ml/hr. The dilution coefficient was D=0.087. During the following four days the flow rate of medium was sharply increased to 50 ml/hr. The dilution coefficient reached D=0.25.

The culture was grown for three days prior to starting the flow of medium through the vessel. During the duration of the whole experiment the mycelium was strongly basophilic, uniform in thickness, and possessed of long hyphae. The hyphae were straight, with hardly any lateral branchings. These properties characterize a young culture. Thin, autolyzed, weakly basophilic hyphae were either completely absent, or occurred very rarely.

A more detailed observation showed mainly two types of hyphae in the visual field: hyphae with a homogenous protoplasm, and those with a changed intracellular structure. We noted, moreover, that with a lower speed of medium flow the homogenous hyphae predominated. As the flow was increased, the hyphae developed a finer granulation, formed segments, and a partially vacuolated protoplasm * (Fig. 3). In the course of the 9-10 day process the pH of the medium varied but little, staying within 6.6-6.8. Antibiotic formation (Table 2) reached 20 billion conditional units by Staphylococcus aureus assay.

Third Series of Experiments. In these experiments we used an original, old culture of A. lavendulae with polymorphous hyphae. Prior to starting the flow of fresh medium, the culture was grown for a long time – five days, i.e., a longer period than in the preceding experiments. Prior to the introduction of medium, the 5 day old mycelium was greatly widened, had short hyphae fragmented into sections or else the protoplasm congregated into roundish formations; along with these hyphae we encountered autolysed, weakly basophilic strands. At this time we started a fresh medium stream at the rate of 25 ml/hr, D=0.120. Carbohydrate concentration of the medium was decreased from 2.5% to 1%. The experimental period was about 10 days (Fig. 4 and Table 3).

As fresh medium was introduced into the culture vessel, the culture, on the whole, was significantly rejuvenated as the process progressed. There appeared, in the visual field, many of the usual normal hyphae with an occasionally interrupted basophilic protoplasm. In these experiments, however, we observed a characteristic presence, throughout the whole period, of short, broadened hyphae. In some of these the protoplasm had lost its basophilic property and become fragile; we en-

* It is as yet difficult to explain exactly what factors influence or cause protoplasmic changes in the hyphae, especially since this question has not yet found a complete explanation even with static cultures. Undoubtedly, these protoplasmic changes – differentiation, loss of basophilic properties, formation of spherical basophilic aggregates, protoplasmic vacuolation – may be caused by a great variety of physico-chemical factors during growth. In earlier experiments, using corn-extract medium, we observed the appearance of typical protoplasmic differentiation when the flow rate of medium was sharply decreased.

TABLE 4. Prolonged Cultivation of A. lavendulae at Low pH Levels and with Interrupted Aeration, without Antibiotic Formation (average rate of fresh medium stream – 8-16 ml/hr)

Indicators	Culture fluid before flow initiation	Duration of process, days												
		1–13	4–6	7–9	10–12	13–15	16–18	19–21	22–24	25–27	28–30	30–33	34–35	37–38
Activity in conditional units	400	2200	25 700	29 800	102 400	500	200	0	0	0	300	400	0	0
Culture fluid pH	5.5	5.5	5.7	5.6	5.5	5.6	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.6
Biomass, g/100 ml	0.46	0.23	0.43	0.34	0.38	0.35	0.32	0.38	0.37	0.51	0.33	0.44	0.22	0.31

countered very thin hyphae and also some with a vacuolated protoplasm. In this case we could see a great variation in hyphal structures. The culture, however, was capable of intensive antibiotic biosynthesis. Antibiotic yield attained the level of $59 \cdot 10^{12}$ conditional units (Table 3).

Further, we were interested to observe morphological mycelial changes (Fig. 5) in those cases where antibiotic was not formed (Table 4). We often encountered processes in which antibiotic biosynthesis did not occur. The cause for this was various factors: interruption of aeration, unfavorable temperature, poor quality of corn extract, and other factors as yet unknown. Here we shall not analyze the whole material, but rather concentrate on the following example. The continuous cultivation lasted 38 days. The culture was grown as usual, first in static conditions in the vessel for 2.5 days, after which the fresh medium flow was initiated. Rate of the flow was 8-16 ml/hr, D = 0.04-0.08. In this experiment we used a different batch of corn extract, and the medium flowed into the vessel without prior treatment with alkali, as a result of which the culture fluid was constantly maintained at a low pH level (5.5). Observations on the culture and its antibiotic activity indicators are presented in Fig. 5 and Table 4. In these conditions, as we see, the mycelium was not uniform; there was a great variation in the hyphae. Among the usual young hyphae of phases II and III there constantly occurred a large number of long and short broadened hyphae (with swellings), and also thin, fragmenting, weakly basophilic hyphae and frankly autolyzed fragments. Consequently, conditions for rejuvenation of this culture were outright unfavorable. We noticed that greatly broadened and shortened hyphae occur, likewise, under the influence of a low pH. Pirt (1959) noted this phenomenon in fungi.

When a culture of A. lavendulae was maintained in a state of active growth, there always occurred, to some degree, variations in intracellular hyphal structure (later this was found to hold true for other actinomycetes as well).

Characteristics of the actinomycete developmental cycle depended on the initial culture (before the streaming), which was employed, in these experiments, in various phases of the culture's development; on the pH of the medium; and on other physico-chemical factors. Changes in these conditions led to the appearance of hyphae varying in their physiological state from the general mycelial mass.

With prolonged cultivation in the upper part of the vessel, an aggregation zone consisting of particles of medium with mycelium appeared over the liquid surface. This zone appeared several days after streaming was initiated. The aggregates partially fell back into the culture fluid, which fact likewise led to the formation of hyphae varying from the general mycelial mass in the medium itself.

Antibiotic biosynthesis in A. lavendulae (as was shown in earlier works) proceeds both in the first and in the second phase of the process. Consequently, when the

one-step scheme is employed with sufficiently high rates of medium flow, we find high antibiotic yields even in the first culture vessel.

Aureomycin snythesis in A. aureofaciens is an example of the two-phase process – therefore, the rate of antibiotic formation (using the same rates of medium flow as for A. lavendulae) is considerably lower in the first step, during active growth of the culture, than in the second. We were primarily interested in the growth and development of A. aureofaciens in a continuous medium. We therefore present below some morphological observations made while maintaining the mycelium in an intensive growth phase, when antibiotic biosynthesis was insignificant. We used a medium containing glucose, NH_4NO_3, corn extract, $CaCO_3$, and NaCl; the pH of the medium was 7.0. The process was conducted in the same glass vessels used for cultivating A. lavendulae. After growing the culture in static conditions, we connected the medium stream. Rate of flow was 10-20 ml/hr, i.e. D=0.05-0.10.

The streaming period was 7 days. Changes in the culture during streaming cultivation are presented in Fig. 6.

We used an aging culture for the experiment. The hyphal plasm had already lost its basophilic properties, it contained large volutin granules, the mycelium fragmented into separate sections – i.e., the starting culture varied considerably in morphology from normal young hyphae. In spite of this, we observed significant changes in the culture upon as little as 27 hr of continuous cultivation. Basophilic hyphae with a homogenous or segmented protplasm dominated the visual field, this mycelial type being characteristic of a young culture in its active growth period. This condition of the culture was maintained for over 5 days in our experiment. An insignificant amount of aureomycin, in the range of 20-182 units, was formed during this period. Thus, our experiments demonstrated that an A. aureofaciens culture, likewise, can be cultivated in a streaming medium for long periods.

SUMMARY

1. Observations have been carried out on the morphology of two actinomyces species, viz., Actinomyces lavendulae and Actinymyces aureofaciens, grown in a streaming medium.

2. It proved possible to keep A. lavendulae and A. aureofaciens in the phase of intense growth throughout the cultivation period (840 and 172 hr, respectively). The bulk of the mycelium exhibited features appropriate to young hyphae. Rejuvenescence of the abnormal aging culture of A. aureofaciens preliminarily grown under stationary conditions could be achieved by a continuous supply of fresh nutrient medium and elimination of the culture fluid.

3. In Actinomyces cultivated in a streaming medium hyphae of a different physiological age are always revealed in the culture.

4. The effect of certain physico-chemical factors was demonstrated, such as pH, quality of the corn extract, rate of supply of the culture fluid, etc., on the appearance of hyphae of different age in a streaming medium.

LITERATURE CITED

M. N. Bekhtereva and I. G. Kolesnikova, 1959, Dokl. AN SSSR, Vol. 127 (5): 1114.

N. A. Krasil'nikov, 1958. Microbial Antagonism and Antibiotic Substances. The "Soviet Science" Press, Moscow.

I. C. Rogotkin, 1958. Acetone-butyl Industry Technology, page 135.

I. Malek, 1956. Microbiology 25 (6): 659.

N. M. Neronova, 1959. Tr. Inst. Microbiol., Vol. 6, 255.

N. M. Neronova and N. D. Ierusalimskii, 1959. Microbiology, Vol. 28 (5): 647.

S. I. Pirt and D.S. Callow, 1959. Nature 184, No. 4683, 307.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

REFLECTION OF PLANE-POLARIZED LIGHT BY CELLS OF THE AERIAL MYCELIUM OF Actinomyces violaceus 829

L. V. Kalakutskii

Institute of Microbiology, Academy of Sciences, USSR
Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 409-413, May-June, 1961
Original article submitted January 12, 1961

The reflection of incident light is affected even by very thin films (Rothen [1956]). Hence, where possible, the application of reflected light to the study of various biological membranes and films is desirable. The use of polarized light provides greater opportunities.

For instance, incident polarized light was effectively used to determine the anistropy parameters of the erythrocyte membrane so that its thickness could be established (Pfeifer [1949]). The employment of microscopy in reflected light in an investigation of the surface structure of the aerial mycelium of actinomycetes (Krasil'nikov and Kalakutskii [1959]; Kalakutskii and Sokolov [1961]; Kalakutskii [1961]) provided additional information about its submicroscopic structure. Here we will merely note the fact that certain regions of this surface appeared bright with crossed polaroids, and hence its optical anisotropy was inferred.

The present paper is devoted to a more thorough study of the anisotropic properties of the surface of the aerial mycelium.

METHOD

We used heat-fixed preparations — prints of a five-day-old culture of Act. violaceus 829 on potato agar. Details of the cultivation of the organism and the preparation of the specimens were given in earlier papers (Kalakutskii and Sokolov [1961]; Kalakutskii [1961]).

All the observations were made with the MBI-6 microscope, epi-objective 40×. This microscope has no attachments for the quantitative study of anisotropy parameters (compensator, devices for reading off angles of rotation of polarizer and analyzer, etc.). It is furnished only with a rotating polarizer mounted close to the field diaphragm of the opaque-illuminator and an analyzer inserted in the path of the rays reflected by the object. During the work the analyzer was rigidly fixed in one position.

The plane of the vibrations transmitted by the analyzer and polarizer was determined by the simple method described by Oster [1955]. A beam of light reflected from glass at approximately Brewster's angle was observed through the polaroid. By rotation of the polaroid the positions of maximum extinction of the observed beam were obtained. In this case the plane of polarization of the polaroid is perpendicular to the plane from which the light is reflected. The directions found were marked by pencil on the polaroid holder. The control consisted of observation in the microscope of an isotropic surface (glass) in the position of maximum extinction with the polarizer and analyzer in position. In this case the planes of polarization of the analyzer and polarizer were mutually perpendicular. In subsequent work we arranged that the plane of polarization of the polarizer would be horizontal in the crossed position.

To avoid extra light reflections in the optical system of the microscope we used a monocular attachment, rather than a binocular, for the observations.

The preparation was rotated by the usual rotating biological stage with a cross-shaped preparation holder.

RESULTS

If the polarizer is rotated close to the crossed position with the analyzer in position the variously oriented cells in the preparation reflect the incident plane-polarized light with different intensity depending on the orientation of its plane of polarization. One of these experiments is shown in more detail in Figs. 1-9. In this case we chose for observation two cells lying at approximately 90° to one another. When the polaroids were crossed and the field was completely extinguished each of these cells made an angle of approximately 45° with the plane of vibrations of the polarizer (Fig. 9).

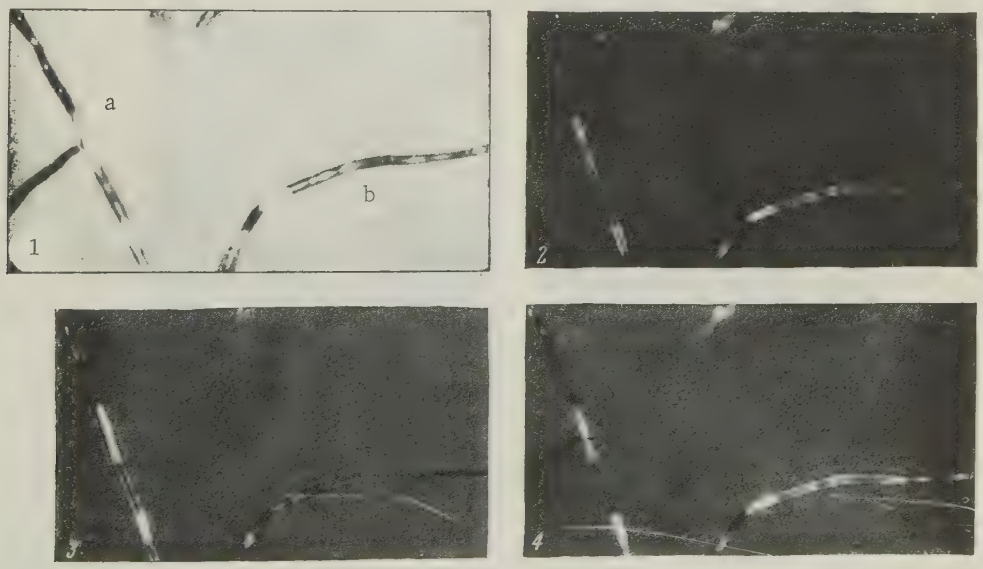
Figure 1 shows the chosen cells photographed in normal incident light (without polarizer and analyzer). Parts of the cells display various interference colors (photograph on black-and-white film). Figure 2 shows the appearance of the same cells with crossed polaroids. The surface of the cells appears bright against a completely dark background. In this preparation the direction of the plane of polarization of the polarizer, as we indicated above, lies along the bisectrix of the angle formed by the cells (i.e., from the upper right to the lower left corner of the figure). We denote the cell lying approximately vertically on the photograph by the symbol a,

and the cell lying horizontally by the symbol b. We now rotate the polarizer from the crossed position to the right (clockwise direction) through several degrees. This gives us the effect illustrated in Fig. 3. The field background becomes brighter (the polaroids are no longer in the crossed position). There is a marked decrease in the intensity of light reflected by cell b (the cell is "extinguished"). A different picture is obtained when the polarizer is turned leftward from the crossed position (anticlockwise: Fig. 4). The background in this case is again a little brighter than in the crossed position, but the intensity of light reflected by cell b is not reduced now, whereas the intensity of the light reflected by cell a is reduced.

To show that the different reflection by cells a and b of incident plane-polarized light when the orientation of its plane of polarization changes is due to this orientation and not to individual properties of the cells a and b, we need only rotate the preparation through 90° without changing the position of the polarizer and analyzer. In this case (Figs. 5-8), clockwise rotation of the polarizer from the crossed position causes the "extinction" of cell a, and anticlockwise rotation of the polarizer causes a reduction in the intensity of light reflected by cell b.

From the results of this and similar experiments we worked out a scheme (Fig. 9). Here AA is the plane of polarization of the analyzer, PP is the plane of polarization of the polarizer, a and b are cells lying at an angle of 90° to one another and at an angle of 45° to the plane of polarization of the polarizer. The essence of the described effect is that rotation of the polarizer from position PP to position P'P' (denoted by broken line) causes the extinction of cell a. On clockwise rotation of the polarizer, cell b is extinguished.

To explain and to make further use of the described effects we will turn to some of the known laws governing the reflection of polarized light from surfaces of anisotropic bodies (Hallimond [1953]). For such surfaces we can distinguish two mutually perpendicular, so-called principal, directions. The indices of reflection of rays polarized in these directions usually differ. When a plane-polarized beam incident on such a surface is reflected its plane of polarization is rotated. As a result, light of a particular intensity is transmitted by the analyzer and can be seen by the observer. (Actually, the situation is complicated by the occurrence of the so-called elliptical polarization, the effect of which is not analyzed here.) There is a general rule, easily deduced and important in our case: The plane of polarization of the incident light after reflection is rotated in the direction in which the major index of reflection is oriented. If the analyzer or polarizer is turned through an angle equal to that turned through by the plane of polarization of the incident beam after reflection, then at the point of compensation we obtain the position of extinction of the anisotropic object. In this case, of course, the analyzer must be rotated in the same direction in which the plane of polarization of the incident light is rotated after reflection, and the polarizer must be rotated in the opposite direction. In our case (Fig. 9) we require the solution of the converse problem: Knowing the direction in which the polarizer must be turned to obtain compensation (point of "extinction" of object), we have to determine how the directions with different indices of reflection



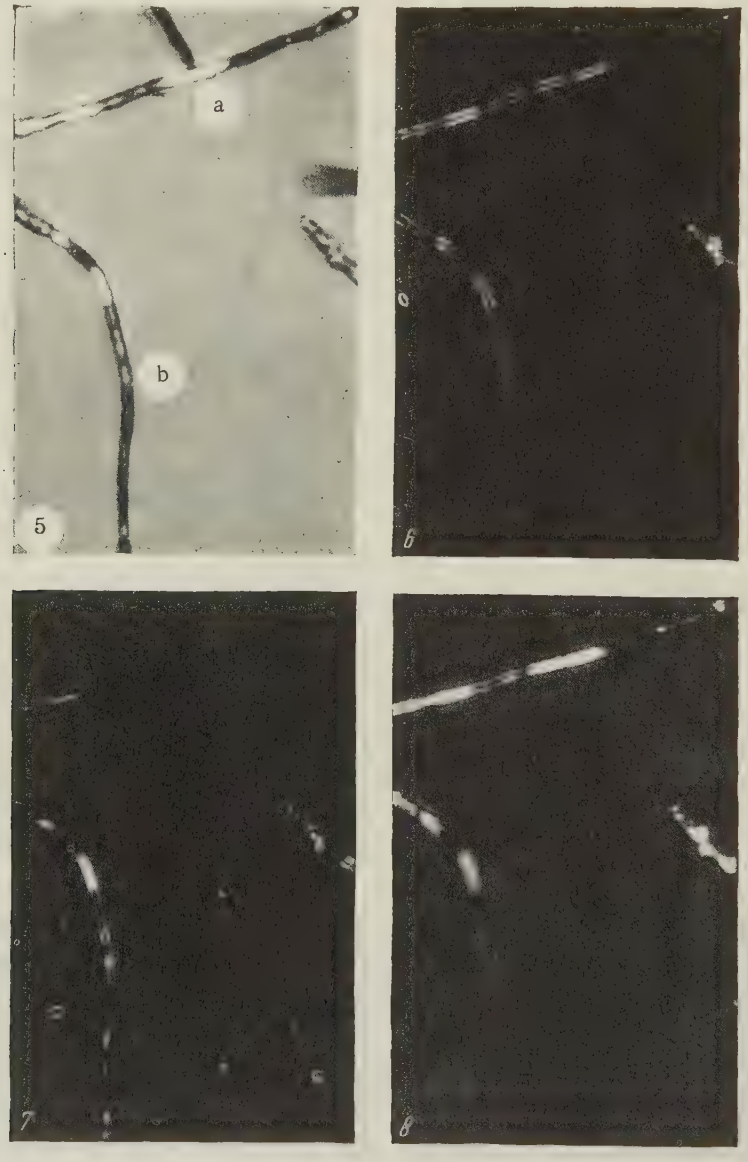
Figs. 1-4. Cells of aerial mycelium of A. violaceus strain 829. Print preparation. 1800×. Fig. 1. View in incident unpolarized light (polarizer and analyzer removed). Fig. 2. View in incident plane-polarized light (crossed polaroids). Fig. 3. The same as in Fig. 2, but with polarizer turned clockwise from crossed position. Fig. 4. The same as in Fig. 2, but with analyzer turned anticlockwise from crossed position.

are oriented on the surface of the object. (That these indices are different is indicated by the very fact of rotation of the plane of polarization by cells a and b, and also by the fact that cells lying at a right angle to one another behave differently in this respect.)

Let the surface of cell a in our scheme possess different indices of reflection, oriented along and perpendicular to the long axis of the cell. Cell a is extinguished when the polarizer is rotated anticlockwise through some angle e. Hence, when the plane-polarized light is reflected from the surface of cell a, the plane of polarization of this light is rotated through the same angle e, but in a direction opposite to that in which the polarizer had to be rotated from the crossed position to the point at which the extinction of cell a was observed. Since the plane of polarization after reflection is rotated in the direction of the major index of reflection, it follows that in cell a the direction of the major index of reflection is oriented perpendicular to its long axis.

A similar argument for cell b leads to the same conclusion – the direction of the major index of reflection of its surface is perpendicular to the long axis of the cell.

This was shown for all cells of the aerial mycelium of A. violaceus in numerous preparations.



Figs. 5-8. The same cells as in Figs. 1-4, but with preparation turned through 90°, and orientation of plane of polarization of polaroids in crossed position as before. Fig. 5. View in unpolarized light. Fig. 6. View with crossed polaroids. Fig. 7. Polarizer turned clockwise from crossed position. Fig. 8. Polarizer turned anticlockwise from crossed position.

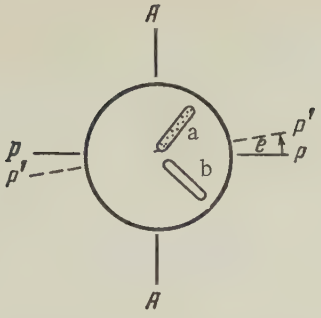


Fig. 9. Scheme. Explanation in text.

Thus, here we have a simple qualitative method of determining the orientation of the directions of the different indices of reflection on the cell surface. It is sufficient to choose two cells (two are taken for comparison) at approximately a right angle to one another and at an angle of 45° to the plane of polarization of the polarizer. The polarizer is then rotated near the crossed position and the positions of "extinction" of the cells are noted. From the direction in which the polarizer has to be rotated the orientation of the directions of the different indices of reflection can be determined from the above argument.

The theory of the change in the plane of polarization on reflection has been worked out for polished surfaces for the case where the incident beam is normal to such surfaces. To what extent does the surface of a heat-fixed microbial cell, particularly the cell of the aerial mycelium of an actinomycete, correspond to such a surface ? If we assume that the cross section of this cell resembles that of a flattened bell, as Finkelstein and Bartholomew [1958] did for Bacillus subtilis, we can infer that such an analogy is valid with certain limitations.

If for reflected light we employ the terminology used (Frey-Wyssling [1953]) for transmitted polarized light, we can say that since the major index of reflection is oriented perpendicular to the long axis of the cells of the aerial mycelium, the latter possess negative bireflection. As is clear from the above account, the sign of rotation of the plane of polarization on reflection is in question, and not the sign of the path difference (retardation of beams) with different indices of reflection. For transparent, nonabsorbing crystals it is believed (Hallimond [1953]) that the distribution of the indices of reflection corresponds to the distribution of the refractive indices directly proportional to them in value. If we accept this for the strongly reflecting regions of the aerial mycelium of the actinomycete, we can make certain further assumptions about the surface structure of these regions.

According to our observations, the cells of the aerial mycelium of the actinomycete A. violaceus display the property of optical anisotropy when examined in transmitted light with crossed polaroids. This property, however, is revealed only when the cells are examined in air. When another immersion medium (oil, water) is used the property of anisotropy in transmitted polarized light is not observed. From this we can assume the presence of form birefringence (texture). If the distribution of the indices of textural birefringence correspond to the distribution of the indices of reflection, then the latter suggests the existence of a composite body with stratified texture, as Wiener [1912] postulated.

In conclusion the author expresses his thanks to Prof. N. A. Krasil'nikov for guidance in the present work.

SUMMARY

1. The surface of the cells of the aerial mycelium of Actinomyces violaceus is characterized by different indices of reflection for incident plane-polarized light.

2. A simple qualitative method of determining the orientation of the direction of the major index of reflection is proposed.

3. The direction of the major index of reflection, determined by this method, is perpendicular to the long axis of the cell.

LITERATURE CITED

L. V. Kalakutskii, 1961. Mikrobiologiya 30: 267.

L. V. Kalakutskii and A. A. Sokolov, 1961. Mikrobiologiya 30: 67.

N. A. Krasil'nikov and L. V. Kalakutskii, 1959. Doklady Akad. Nauk SSSR 129: 1165.

H. Finkelstein and J. Bartholomew, 1958. Stain Technol. 33, 177.

A. Frey-Wyssling, 1953. Submicroscopic morphology of protoplasm. Elsevier Publ. Co., 2-d ed., Amsterdam.

A. Hallimond, 1953. The polarizing microscope. Cook, Thoughton, a. Simms, Ltd., York, Engl. 2-d ed.

G. Oster, 1955. Birefringence and dichroism. In: Oster G. and Pollister A. Eds. Physical techniques in biological research. Acad. Press Inc., N. Y., 1.

H. Pfeifer, 1949. Kolloidzeitschr. 113, 6.

A. Rothen, 1956. Surface film and surface tension technique. In: Oster G. and Pollister A. Eds. Physical techniques in biological research. Acad. Press Inc., N. Y., 2.

O. Wiener, 1912. Abh. sächs. Ges. Wiss. 33, 47 (cited by A. Frey-Wyssling, 1953).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

PHAGE OF THE THERMOPHILIC Micromonospora vulgaris

N. S. Agre

M. V. Lomonosov Moscow State University

Translated from Mikrobiologiya, Vol. 30, No. 3, pp. 414-417, May-June, 1961
Original article submitted September 30, 1960

In recent years, the phage phenomenon has been attracting the attention of many investigators. Most of the work is devoted to the phages of mesophilic microorganisms. There are few data in the literature concerning the phages of thermophilic microorganisms, and we have encountered no information at all on thermophilic actinophages. In the present communication, the results of a study of phages which were isolated by us from lysogenic cultures of the thermophilic actinomycete – Micromonospora vulgaris – are reported.

While examining the soils of Pamir, we isolated cultures of thermophiles of the genus Micromonospora; we related 90 of the cultures to M. vulgaris (Krasil'nikov, 1949).

When some of the cultures of this species were plated on potato-corn agar (PCA, or peptone-corn medium, consists of 10 g of starch, 5 g of NaCl, 0.5 g of $CaCl_2$, 5 g of peptone, 5 g of corn extract, 20 g of agar, and 1 liter of tap water) and fish-extract medium, we observed the appearance on the surface of the background of negative colonies characteristic of phage. The negative colonies on backgrounds of different cultures differed in size and shape (table).

Cultures No. 373, 800, 1211, and 1338 of M. vulgaris were clearly lysogenic. These cultures served as the material for the isolation of the thermophilic phage. Negative colonies were most frequently observed on potato-corn medium, which was therefore the medium that we selected for further work.

To isolate the phages, we used the methodology described in the works of Rautenshtein (1957) and of Khavina and Rautenshtein (1958). The first stage of our work consisted of the detection, among the 90 cultures of M. vulgaris cultures that we had isolated, of strains (indicator) sensitive to the phages contained in lysogenic cultures No. 373, 800, 1211, and 1338. For this purpose, agar blocks of 24-hr cultures of the lysogenic strains were placed on freshly-prepared backgrounds of all 90 cultures, plated on petri plates of potato-corn medium. Incubation was carried out at 55 C for 16-20 hr.

The appearance of sterile zones around the superimposed blocks was noted on the backgrounds of most of the cultures. A small piece of agar was taken from these sterile zones with a loop; it was placed on a freshly-prepared background of the same culture, and spread over the entire surface of the medium with a spatula. In all cases where sterile zones arose due to phage, its multiplication was observed on the test-culture background.

Thus, a number of indicator cultures were found on which phage reproduction was further carried out. Phage from M. vulgaris culture No. 373 multiplies on M. vulgaris culture No. 395, phage from culture No. 800 – on culture 575, phage from culture 1211 – on culture 679, and phage from culture 1338 – on 402.

In order to purify the phages isolated, each of them, in turn, was isolated from a single negative colony. After purification, the phages were propagated on potato-corn broth (pH 7), after which the latter was filtered through a Seitz filter.

In this manner, we isolated four phages from the lysogenic cultures mentioned above. The isolated phages were further propagated on indicator cultures in liquid peptone-corn medium on a shaker at 55 C. Under these conditions, we obtained phage lysates with titers of 10^8 or 10^9.

The temperature optimum for the lysis of the isolated phages coincides with the optimum for the growth

Size of Negative Colonies of the Phages under Investigation as a Function of the Test Culture

Phage No.	Test culture		Negative colony size, mm
	Micromonosrora vulgaris		
373	The same	575	0.1—0.7
	»	679	1—3
800	»	575	0.1—0.7
	»	679	1—3
1211	»	575	0.1—0.7
	»	679	1—3
1338	»	575	0.1—0.7
	»	679	1—3

of M. vulgaris. On peptone-corn agar, lysis of the mycelium at 55 C was observed after 5-7 hr of incubation. At the start of incubation, in 4-5 hr after inoculation, a thin, nearly transparent film of mycelium appears on the surface of the agar in both experimental and control plates. After 7-8 hr, aerial mycelium starts to form in the control. In the experimental plate at this time, lysis of the mycelium that had grown occurs, and the surface of the agar becomes exposed. Secondary growth appears in the experimental plates 10-11 hr from the start of incubation.

The growth appears either in the form of discrete colonies, or all the initially lysed area becomes completely overgrown by mycelium which resembles the mycelium of a normal culture in its cultural characteristics. After 18-20 hr, it is sometimes difficult to distinguish the experimental plate from the control.

During reproduction of the phages in liquid media, slight growth appears at the start of the cultivation of the culture with phage, both in experimental and in control flasks. By the fifth or sixth hour of incubation, clearing of the medium is observed in the flasks with phage as the result of phage lysis. In 9-10 hr, secondary mycelial growth begins in the experimental flasks, and after 12 hr of incubation, they cannot be distinguished from the control flasks in intensity of growth. We made subcultures from colonies of secondary growth to agar slants. Ten cultures of secondary growth were isolated from the four indicator cultures used in the experiments. They did not differ from the original forms in morphological and cultural properties; the character of spore formation and growth on liquid and agar media was the same in both.

Cultures from secondary growth differed from the original ones by slower growth. Whereas in the original cultures mycelium visible to the naked eye was already apparent in 4-5 hr after inoculation of peptone-corn agar and aerial mycelium was visible in 7-8 hr, the variants obtained from secondary growth formed visible submerged mycelium after 10-12 hr and aerial mycelium after 24-30 hr of incubation at 55 C. All of the secondary growth variants isolated proved to be resistant to the phages affecting the original cultures.

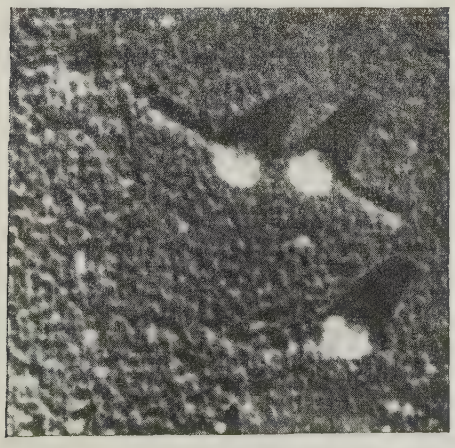


Fig. 1. Electron photomicrograph of phage No. 375.

A study of the isolated phages with the aid of an electron microscope showed that they are similar to one another in morphology and size. They all have a spermatozoidal shape (Fig. 1).

The length of the head is 84 mμ, the width of the head – 72 mμ; the length of the tail – 120 mμ, its width – 20 mμ.

All of the indicator cultures were found to be sensitive to Na citrate. They failed to grow when its concentration in the medium was 2%. At a concentration of 1.5%, barely noticeable growth was observed, 1% Na citrate inhibited growth slightly, while 0.5% and 0.25% had no effect on culture growth. Phage lysis was completely inhibited in the presence of 1% Na citrate in the medium.

At a concentration of 0.5%, phage lysis was lessened, whereas at 0.25%, phage lysis proceeded in the same manner as in the control.

The lytic properties of all four of the isolated phages were checked on mesophilic and thermophilic representatives of the genera Micromonospora and Actinomyces.

For this purpose, mesophilic actinomycetes were plated as a background on peptone-corn agar with glucose and peptone agar with starch, mesophilic micromonosporas were plated on potato medium, and thermophilic actinomycetes and micromonosporas were plated on peptone-corn agar with starch. A drop of phage lysate was then placed on the backgrounds of test cultures.

Twenty-five mesophilic species of the genus Actinomyces obtained from various collections were used for the experiment: Actinomyces viridichromogenes, Actinomyces variabilis var. roseolus, Actinomyces scabies, Actinomyces globisporus var. roseus, Actinomyces olivaceus, Actinomyces violaceus, Actinomyces levoris, Actinomyces rutgersensis, Actinomyces daghestanicus, Actinomyces odorifer, Actinomyces caeruleofuscus, Actinomyces badius, Proactinomyces albus, etc.

Among the mesophilic micromonosporas at our disposal were Micromonospora coerulea, Micromonospora fusca, and nine cultures of Micromonospora sp. obtained from Krasil'nikov's laboratory. Thermophilic test cultures of the genera Actinomyces and Micromonospora were taken from our collection. The thermophilic actinomycetes were represented by ten cultures belonging to a single species according to all characteristics, but the thermophilic micromonosporas were represented by 20 strains that clearly fell into two groups. All of the thermophilic cultures were isolated by us from Pamir soils.

All of the actinomycete and micromonospora cultures tested, both thermophilic and mesophilic, were found to be resistant to all four of the phages tested. A

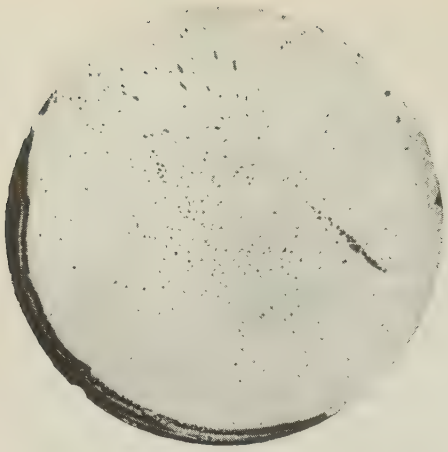


Fig. 2. Negative colonies of phage 800 on culture No. 575.

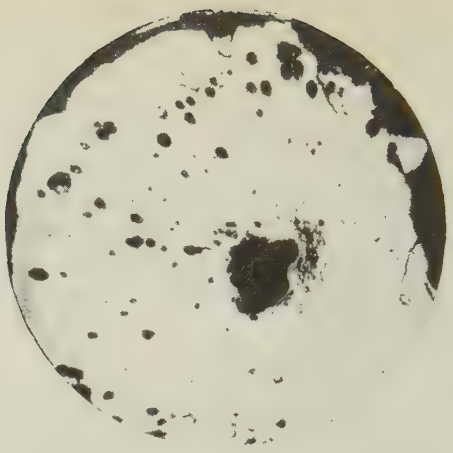


Fig. 3. Negative colonies of phage 1211 on culture No. 679.

check of the effect of the phages on 30 strains of vulgaris showed that all of these strains of M. vulgaris were sensitive to phages No. 373, 800, 1211, and 1338 to the same extent.

A study of the morphology of the negative phage colonies enabled us to establish that phage No. 1211 differs from the rest in this characteristic.

Phages No. 800, 373, and 1338 formed predominantly small negative colonies measuring 0.1-0.7 mm in diameter (Fig. 2), but phage No. 1211 was larger – 1-3 mm (Fig. 3). However, further study of this question showed that, on M. vulgaris culture No. 595, all four phages produced small, pin-point colonies measuring 0.1-0.7 mm in diameter. On culture 679, the negative colonies were larger – from 1 to 3 mm (table).

The present work was carried out under the direction of Prof. N. A. Krasil'nikov, for which we offer our deep appreciation.

SUMMARY

Four phages have been isolated from lysogenic cultures of the thermophilic micromonospora (Micromonospora vulgaris) which proved identical to one another and specific for the cultures of this species.

The optimum temperature is 55 C for lysis caused by these phages. The phages have a sperm-like shape. The head is 72× 84 mμ, and the tail 20×120 mμ.

LITERATURE CITED

1. N. A. Krasil'nikov, 1949. Determinative Key of Bacteria and Actinomycetes [in Russian] (Izd. AN SSSR, 1949).
2. Ya. I. Rautenshtein, Mikrobiologiya 26, No. 5 (1957).
3. É. S. Khavina and Ya. I. Rautenshtein, Mikrobiologiya 27, No. 4 (1958).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

EFFECT OF AERATION ON HETEROFERMENTATIVE LACTIC ACID FERMENTATION WITH RESPECT TO ACIDITY OF THE MEDIUM

N. N. Grechushkina

Soil Biology Faculty, M. V. Lomonosov Moscow State University
Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 418-425, May-June, 1961
Original article submitted June 23, 1960

Data obtained in the laboratory of V.N. Shaposhnikov demonstrate that aeration changes the ratio of products of heterofermentative lactic acid fermentation in the direction of an increased yield of the more oxidized compound, acetic acid. The more reduced compounds, ethyl alcohol and lactic acid, are generally accumulated under anaerobic conditions (Shaposhnikov and Semenova, 1949; Rabotnova, 1957; Feofilova, 1958; Tarasova, 1958). However, there is very little information regarding the action of aeration accompanied by simultaneous changes in pH of the medium. In a previous investigation (Grechushkina, 1961), the significance of pH was made clear in the growth of Lactobacterium pentoaceticum from kvass wort and the progress of its heterofermentative lactic acid fermentation. In this paper the effect of aeration was studied on the character of the L. pentoaceticum fermentation with respect to the acidity of the medium.

The experiments were carried out at 5.5 Balling in wort with $MnSO_4$ (110 mg/liter) at 37 C. To hamper air access to the cultures, they were incubated in test tubes with a diameter of 2 cm containing 35 ml of medium. The height of the layer of medium was 12 cm. The aerated cultures were incubated in Winogradsky flasks with the same volume of medium, the height of the medium being 1.5 cm. The inoculum, a day-old culture on wort with chalk and wort solids, was introduced at a level of 1% of the volume of the medium; the initial cell count in the medium was 20-30 million cells/ml. Determination of the fermentation products was as described in a previous communication (Grechushkina, 1961). The fermentation products were expressed in millimoles/liter; sugar, in millimoles of triose/liter; and the harvest of bacteria, in millions of cells/ml medium. The data presented are individual examples taken from several similar experiments, calculated on the basis of control determinations in sterile wort.

RESULTS

Action of aeration on the ratio of fermentation products at optimal acidity of the medium (pH approximately 5.0). Previous experiments showed that slightly acid conditions were optimum for the development of L. pentoaceticum (pH about 5.0; this was achieved by the addition of chalk to the wort during the fermentation process. The dynamics of fermentation on wort and chalk under semi-anaerobic conditions are presented in Fig. 1. The basic products of fermentation were lactic acid and alcohol. At 72 hr the ratio of lactic acid to alcohol=3/2. Acetic acid was accumulated in small quantities. The ratio of fermentation products during bacterial growth on wort and chalk medium under aerobic conditions is shown in Fig. 2. Comparison of these results with results of the semi-anaerobic experiments shows that aeration markedly inhibited growth and fermentation. The maximal harvest of cells during aeration was almost 15 times less, about 200 million/ml. Even after 24 hr, fermentation was essentially at a standstill. A large residue of sugar accompanied the low yield of products. At the same time, aeration altered the ratio of fermentation products: the formation of the more reduced product, alcohol, was almost completely suppressed. The yield of lactic acid was greatly curtailed. The formation of acetic acid was relatively less inhibited. Thus, oxygen not only inhibited the growth of the bacteria, but interfered with their metabolism, inhibiting the yield of the more reduced product and increasing the relative yield of acetic acid.

Action of aeration on the ratio of fermentation products under strongly acid conditions (initial pH=5.0-5.5). A strongly acid pH=3.2-3.4 occurring in naturally acidified wort is unfavorable for L. pentoaceticum (Fig. 3). Even under semi-anaerobic conditions growth and fermentation was inhibited and the ratio of fermentation products greatly altered. The main product became alcohol, so that the ratio of lactic acid/alcohol = 1/2. Such an accumulation of alcohol, which is unusually high for a heterfermentative lactic acid fermentation of L. pentoaceticum, is apparently the result of its readily adaptable character. Stabilization of the intermediate fermentation products in the form of neutral alcohol is a consequence of the protection of the bacteria from the excessive acidity of the medium. In order to explain in what manner the influence of aeration on the development of L. pentoaceticum was related to the acidity of the medium, the dynamics of fermentation on naturally acidi-

fied wort under aerobic conditions was investigated. Results, presented in Fig. 4, show that in strong acid also, aeration inhibited the development and fermentation of L. pentoaceticum. The maximal harvest of cells was almost five times lower than under semi-anaerobic conditions on wort without chalk, but were almost the same as aeration on wort with chalk, about 200 millions/ml. The morphology of the cells was changed; they were lengthened. At the same time aeration also shifted the ratio of fermentation products. It could be expected that aeration would curtail the yield of reduced compound, alcohol, and at the same time increase the amount of acetic acid, as was observed in the fermentation with chalk. But it was shown that,as in the aerated fermentation with chalk, the accumulation of

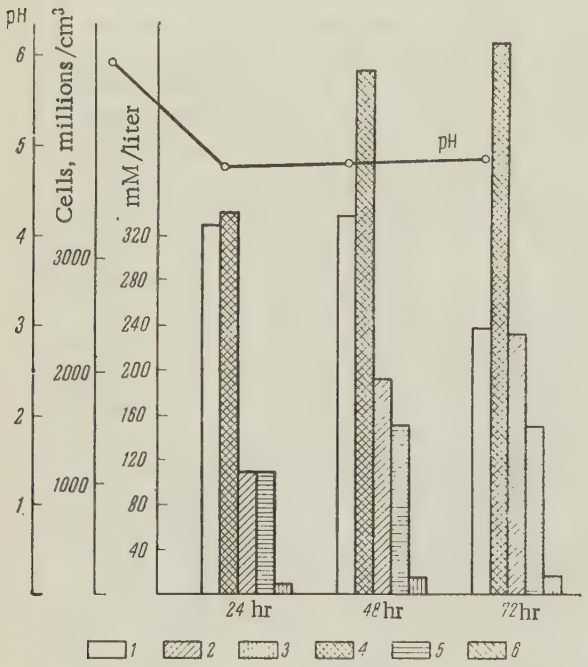


Fig. 1. Dynamics of fermentation under semi-anaerobic conditions on wort and chalk. 1) Cells; 2) lactic acid; 3) acetic acid; 4) sugar (trioses); 5) alcohol; 6) CO_2.

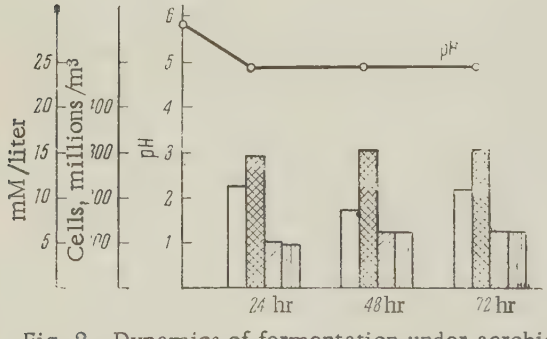


Fig. 2. Dynamics of fermentation under aerobic conditions on wort and chalk (the scale is increased, designations the same as in Figure 1).

acid was stopped very much earlier, at 6-10 hr, and the total yield of acid was equal to approximately 10 mM/liter. The formation of acetic acid was somewhat less than that of lactic acid. The ratio of acids at pH=3.2 and 5.0 were only slightly different. But the reduced product, alcohol, as on unneutralized wort in semi-anaerobic conditions, remained the main product of fermentation, while on aerated wort neutralized with chalk it was not formed at all. Its accumulation paralleled growth and continued even until the formation of acid had stopped. At 24 hr the ratio of lactic acid/alcohol = 1/4-1/5, that is, the yield of alcohol had increased two-fold in comparison with lactic acid. Thus, the effect of aeration on the growth and characteristics of L. pentoaceticum fermentation on wort depend on the acidity of the medium.

In a previous paper we showed that under semi-anaerobic conditions in a strongly acid medium, the carbon moiety of all fermentation products was apparently formed from sugar. The carbon balance of aerated fermentation in strongly acid wort showed that the amount of carbon in the fermentation products significantly exceeded the carbon of the sugar consumed (data were related to 48 hr fermentation) (Fig. 5). Probably under these conditions a large part of the alcohol was formed from non-carbohydrate substances in the wort. We determined the amount of carbon in the carbohydrate and non-carbohydrate portions of 3 Balling wort. The data show that the carbon, exclusive of that contained in sugar, was completely adequate for the formation of the amount of alcohol resulting from fermentation:

	carbon in mg/100 ml
sterile wort (a)	1356.0 (determined by the wet combustion method)
in that amount, sugar (b)	981.4 (at first the total sugar content was determined by the micromethod of Berry, and then converted to mg C)
remaining components of wort (a–b)	374.6
alcohol formed	140.0

In the literature there are indications to the effect that on complex media in certain conditions part of the products of heterofermentative lactic acid fermentation can be formed from non-carbohydrate subtances. Christensen, Albury, and Pedersen (1958) reached this conclusion. Conducting a fermentation balance with L. breve and Leuconostoc mesenteroides on a medium with peptone and yeast extract, they also found a carbon surplus in fermentation products in comparison with the carbon of the utilized sugar.

Wort Solids as a Reducing Agent

During these experiments it was noted that wort with solids gave better growth of L. pentoaceticum than wort without solids. Analysis of the fermentation products during semi-anaerobic fermentation in naturally acidified wort with solids (2% of the volume of the medium)

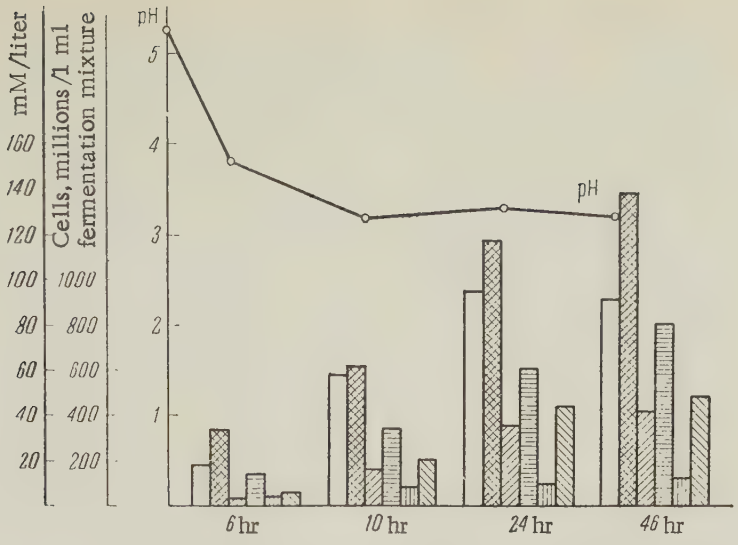


Fig. 3. Dynamics of fermentation under semi-anaerobic conditions in strongly acid medium (scale increased in comparison with Fig. 1).

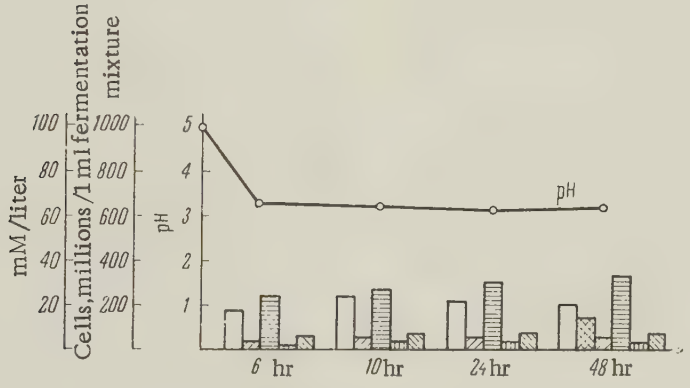


Fig. 4. Dynamics of fermentation under aerobic conditions in strongly acid medium (designations the same as in Fig. 1).

and without solids showed (Fig. 6) that in this case the solids did not alter the ratio of fermentation products. On wort with solids, growth was greater than on wort without solids, and there was a corresponding increase in yield of products and utilization of sugar. The main product was alcohol, which in general is characteristic for L. pentoaceticum fermentation in highly acid wort. If these data are compared with the corresponding fermentation products on wort with solids and without solids in aerobic conditions without chalk, then it appears that in wort with solids, aeration does not essentially affect the growth and ratio of fermentation products of L. pentoaceticum. The solids must then completely exclude the inhibitory action of oxygen. The question then arises, what do the solids do for the bacteria? Solids are proteinaceous and can be used as a supplementary source of nutritive substances. But since we observed good bacterial growth in the presence of solids under aerobic conditions, while without solids growth was inhibited, we hypothesized that solids contained a reducing substance, protecting the bacteria from the unfavorable effects of oxygen of the air. In order to clarify this problem, the oxidation-reduction potential (rH_2) was measured in sterile wort with and without solids. The conditions of the previous experiments (section 2), including temperature regulation, were strictly preserved. The electrodes were submerged directly in the solids. The potential was measured during definite time intervals. At the end of the experiment, the sterility of the medium was

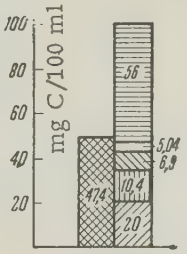


Fig. 5. Carbon balance (strongly acid wort, aeration).

tested by inoculation on meat-peptone agar and by microscopic examination. Results of one of the experiments are presented in Table 1.

On wort with solids the potential was lower than on wort without solids both with free and restricted air access. But in the latter case the reducing property of the solids was much more distinct; a gradual fall in potential was observed in spite of the sterility of the medium. Apparently, the solids in reality contain a reducing agent which takes up oxygen. In this way solids protect the bacteria from the effect of aeration. Thus, the effect of aeration in great measure depends on the content of reducing substances in the medium.

DISCUSSION

Aeration greatly influences the growth and fermentation of L. pentoaceticum on wort. Independent of the acidity of the medium, aeration nearly always markedly inhibits the growth of bacteria. This apparently is explained by the fact that the culture is attracted to reducing conditions of the medium. Measurement of the oxidation-reduction potential during the growth of the cultures with varying aeration showed (Table 2) that such conditions are created when air intake is inhibited. Under aerobic conditions the potential is much higher.

At the same time the presence of oxygen changes the ratio of fermentation products. The character of such changes is determined by the acidity of the medium. At optimal acidity (pH approximately 5.0) aeration, as could be expected, inhibits the yield of reduced compounds (lactic acid and alcohol, which under these conditions are not formed) and increases the relative yield of acetic acid. In strongly acid aerated wort (pH= 3.2-3.4), as in the wort neutralized with chalk, the maximum harvest of bacteria is around 200 million/ml, and the total yield of acid is about 10 mM/liter. Under these conditions the influence of aeration compares with the effect of other unfavorable factors – an acid pH of the medium. The influence of acidity on the character of the fermentation is stronger than the influence of aeration. The bacteria are protected from an excess of acidity by the formation of large quantities of neutral product – alcohol. Under semi-anaerobic conditions, the ratio of lactic acid/alcohol = 1/2. It would be expected that on aeration the yield of reduced product – alcohol – would be maintained. But the ratio of lactic acid/alcohol is equal to 1/4-1/5. The relative yield of alcohol is still increased. This creates the impression that aeration aggravated the unfavorable effect of acidity, compelling the bacteria to produce a reducing, but neutral

TABLE 1. rH_2 of Sterile Wort with and without Solids

Aeration	Medium-wort	Time, hr	pH	Eh, mv	rH_2
Semi-anaerobiosis	With solids	0	5.88	110.95	24.16
		27	5.88	28.6	21.36
		52	5.88	−28.0	19.36
	Without solids	0	5.93	263.0	29.56
		27	5.93	248.5	28.96
		52	5.93	232.0	28.46
Aerobiosis	With solids	0	5.88	220.5	27.96
		27	5.88	206.0	27.46
		52	5.88	186.0	26.76
	Without solids	0	5.93	308.5	31.06

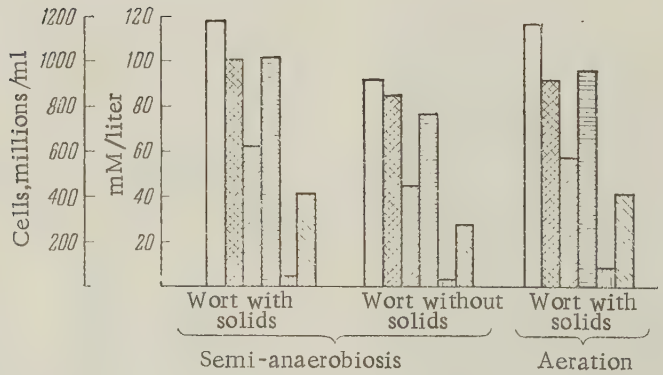


Fig. 6. Growth and ratio of fermentation products of L. pentoaceticum in wort with and without solids (48 hr). At the beginning of the experiment pH=5.48; at the end the pH = 3.3.

TABLE 2. rH_2 in L. pentoaceticum Cultures under Varying Conditions of Aeration.

Aeration	Condition of acidity	Time, hr	pH	Eh (potential), mv	rH_2
Semi-anaerobiosis	Without chalk	0	4.98	288.5	28.56
		5	3.51	149.5	20.62
		23	3.29	—185.0	8.82
		46	3.12	—170.0	8.94
	With chalk	0	5.24	267.0	28.28
		5	4.91	35.0	19.62
		23	4.66	—166.0	12.22
		46	4.73	—172.5	12.06
Aerobiosis	Without chalk	0	4.98	291.0	28.56
		5	3.28	281.0	24.86
		23	3.12	295.0	25.04
		46	3.24	256.5	23.98
	With chalk	0	5.10	314.5	29.70
		5	4.30	345.0	29.10
		23	5.13	326.0	30.12
		46	4.04	317.5	27.68

product, alcohol, in larger quantities than in semi-anaerobic conditions. The fact of the extreme lability of alcohol formation in the L. pentoaceticum fermentation in various conditions of cultivation leads to the supposition that its accumulation in some cases is a specific adaptive reaction of this organism to unfavorable conditions of acidity. Apparently, in strongly acid wort during aeration a large part of the alcohol is formed, not from sugar, but from non-carbohydrate components of the wort. Then the fermentation assumes an unusual distorted form. Thus, the influence of aeration on L. pentoaceticum fermentation is related to the pH of the medium. Not all heterofermentative lactic acid bacteria are as definitely inhibited by oxygen as our strain of L. pentoaceticum. Tarasova (1958), in determining the composition of nutritive media, showed that the growth of L. pentoaceticum isolated from cabbage brine was the same or even higher during aeration than under anaerobic conditions. On a medium with glucose, oxygen was almost without inhibitory effect on the browth of bacteria, but in its presence the yield of acetic acid was greatly increased and the yield of alcohol was reduced. On a medium with pyruvic acid and glucose the formation of acetic acid on aeration was increased, but the accumulation of alcohol was markedly inhibited either under aerobic conditions or with aeration. The influence of aeration on our organism also was shown to be dependent on the composition of the medium. If oxygen greatly inhibits the growth of bacteria and alters the character of the fermentation in wort without solids, the growth and fermentation of L. pentoaceticum on wort with solids is shown to be almost independent of moderate aeration. The harvest of bacteria and the total yield of products on aeration is as high as under semi-anaerobic conditions. The ratio of fermentation products was almost the same. Solids completely exclude the inhibitory effect of oxygen on the progress of fermentation. Determination of the oxidation-reduction potential of sterile media with and without solids showed that the solids contain a reducing agent. They apparently take up oxygen, thereby almost excluding its effect on the metabolism and development of the bacteria.

In conclusion, thanks are extended to Prof. I. A. Rabotnova for the direction of this work.

SUMMARY

1. Lactobacillus pentoaceticum when cultivated on wort is restricted to anaerobic conditions. Oxygen of the air markedly inhibits the growth of the bacteria.

2. Oxygen of the air, besides its inhibitory action, alters the ratio of fermentation products, but its effect depends on the pH of the medium.

3. At optimal acidity (pH=5.0) the relative yield of the oxidized product, acetic acid, is increased, while the reduced product, alcohol, is not formed.

4. At strongly acid values of the pH, occurring in fermentation without chalk, aeration not only does not inhibit, but even increases, the relative yield of reduced product — alcohol.

5. Solids of beer wort possess reducing properties. Introduction of 2% solids in wort almost completely curtails the action of moderate aeration on the growth and metabolism of L. pentoaceticum. Under semi-anaerobic conditions, solids increase the harvest of cells and the yield of product, while not changing their ratio.

LITERATURE CITED

1. N. N. Grechushkina. 1961. Mikrobiologiya 30, Nos. 1 and 2.
2. I. L. Rabotnova. 1957. Role of Physical-Chemical Conditions (pH and rH_2) in the Life Activity of Microorganisms. [In Russian] Izd. AN SSSR, Moscow.
3. N. V. Tarasova. 1958. Mikrobiologiya 27, No. 3.
4. E. P. Feofilova. 1958. "Microflora of Ossetic kefir yeasts." Dissertation, Moscow State University.
5. V. N. Shaposhnikov and V. A. Semenova. 1949. Mikrobiologiya 18, No. 2.
6. M. D. Christensen, M. N. Albury and C. S. Pedersen. 1958. Appl. Microbiol. 6, No. 5, 316.

RELATIONSHIP OF SOME SPECIES OF GASEOUS HYDROCARBON-OXIDIZING BACTERIA TO HYDROCARBONS OF THE PARAFFIN SERIES

Z. P. Telegina

All-Union Scientific Research Geoexploratory Oil Institute, Moscow
Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 426-430, May-June, 1961
Original article submitted October 14, 1960

Methane- and propane-oxidizing bacteria have been used as basic indicator organisms for microbiological search for oil and gas in underground waters and strata.

A large body of factual material representing many years' work carried out at the All-Union Scientific Research Institute for Geological Survey has been accumulated for various oil- and gas-bearing regions on the propagation of these microorganisms(Mogilevskii, 1958, 1958a; Samoilova and Lisitsina, 1958). But there is little data regarding the species composition of these groups of bacteria.

The problem of the utilization of gaseous hydrocarbons by individual species of indicator microorganisms has also been insufficiently studied. The investigations of different authors on this problem when compared show different opinions, with the result that some authors point out the strict specificity of individual species with respect to the utilization of these or other homologues of gaseous hydrocarbons, and the others establish the ability to oxidize a large number of methane homologues. Thus, for example, Methanomonas methanica, according to some authors, was capable of oxidizing only methane and was not able to utilize higher molecular weight hydrocarbons (Sönhgen, 1913; Münz, 1915). Cultures of methane-oxidizing bacteria isolated by other authors utilized not only methane, but ethane and propane also (Hutton and ZoBell, 1949; Strawinskii, 1955; Davis, 1956).

This contradiction leads some authors to conclude that there are some species of microbes capable of oxidizing methane. Other authors explain this phenomenon by altered conditions of the environment. At the present time this problem remains unsolved; further study and accumulation of experimental data are required.

Because of this, we undertook the important study of the species composition and characteristic features of bacteria oxidizing gaseous hydrocarbons. We isolated individual species of bacteria from underground waters and some cores in levels of the gas-bearing areas of the Azov-Kuban depression: Leningrad, Kanevka, Staro Minskaya, Novo Sherbinovskaya, and others. Waters of the Pliocene and Quaternary deposits were the main objects of investigation. Samples for analysis were withdrawn from artesian wells and pits. The depth of the investigated water-bearing levels did not exceed 250 m. The core samples were taken from productive strata of chalk deposits at a depth of 2050-2300 m.

As a result of microbiological analysis of underground waters and cores from these regions we succeeded in obtaining an aggregate of cultures of methane- and propane-oxidizing bacteria, part of which were further purified, and from which pure cultures were finally isolated.

In Table 1, characteristics of water samples are given, from which cultures of methane- and propane-oxidizing bacteria were obtained. It may be seen from the table that pure cultures of hydrocarbon-oxidizing bacteria were isolated from seven samples of water, withdrawn either in the contour or beyond the contour of gas-bearing formations. The water samples were fresh as measured by salt composition and belonged to water of the sodium bicarbonate and sodium sulfate types.

Hydrocarbons were found in all samples of water, with the exception of one sample, No. 28, when dissolved gases were analyzed on a VTI apparatus. Ten pure cultures of bacteria oxidizing methane and propane were isolated from the water samples. The cultures were inspected for purity by microscopic examination and by inoculation on meat-peptone agar (MPA). The presence of uniform colonies on meat-peptone agar and identical rods under the microscope indicated the purity of the culture. They were further subjected to species determination and physiological investigation.

Two strains were isolated in a methane atmosphere: one from the Quaternary layer water, the second from a core sample of chalk deposit. The first strain, as indicated by its morphological and cultural characteristics, belonged to Methanomonas methanica.

The second strain was shown to be Bacterium methanicum (No. 2075). Although both strains were isolated in a methane atmosphere, they behaved differently with respect to hydrocarbons of the paraffin series. The first strain only oxidized methane, the second one utilized hexane and heptane in addition to methane.

TABLE 1. Characteristics of Underground Waters from Pliocene and Quaternary Deposits of Gas Formations in the Azov-Kuban Depression, from which Were Isolated Pure Cultures of Hydrocarbon-Oxidizing Bacteria

No. water sample	Name of the area in which the source of the isolated culture was located	Characteristics of the water sample	Depth of the water-sampling level	Age of the deposit, coning the water-bearing level	Relation to gas-and oil-bearing properties (position of the water sample in relation to the gas-bearing contour)	Content of saturated hydrocarbons in ml/liter according to data on the VTI apparatus	Hydrocarbon in the atmosphere of which the isolated strain grew	Species of microorganism isolated from the sample
6	Kanevka	Well	220	Tertiary deposit	Near the gas-bearing contour	0.9	Propane	1) P. pantotropha 2) Pseudomonas species
8	"	"	240	same	same	1.7	"	P. pantotropha
13	"	"	200	"	In the gas bearing contour	0.7	"	P. caudatus
25	Leningrad	"	200	"	same	2.2	"	Pseudobacterium subluteum
26	same	Pit	5-10	Quaternary deposit	"	2.25	"	Mycobacterium rubrum var. propanicum
28	"	Well	150-200	Tertiary deposit	"	0	"	Mycobacterium carotenum Pseudomonas species
36	Staro Minskaya	Pit	5	Quaternary deposit	"	2.0	Methane	Methanomona methanica

Note: Horizontal lithology—sand.

TABLE 2. Relationship of Bacteria Oxidizing Methane and Propane to Paraffin Hydrocarbons

Culture No.	Microorganism	Cultures first isolated from	Bacterial growth rate in an atmosphere of						
			methane	ethane	propane	butane	pentane	hexane	heptane
36	Methanomonas methanica	CH_4	++	0	0	0	0	0	0
2075	Bact. methanicum	CH_4	+++++	0	0	0	0	+++	++
26	Mycobact. rubrum	C_3H_8	0	0	+++	0	0	+++	++++
28—1	Mycobact. carotenum	C_3H_8	0	0	+	0	0	0	0
28—3	Mycobact. species	C_3H_8	0	0	++	0	0	++++++	+++++
13	Ps. caudatus	C_3H_8	0	+	+	+	+	+	+
25	Pseudobact. subluteum	C_3H_8	0	+	+	+	+	+	+
6—3	Ps. species	C_3H_8	0	+	+	++	+	++	+
6	Ps. pantotropha	C_3H_8	++	+	+++	++++	+++	+++	+
8	» »	C_3H_8	+	+	+++	+++	++	++	+

Note: + signifies that the hydrocarbon was utilized, 0 that it was not.

TABLE 3. Rate of Oxygen Uptake during Respiration of Cultures of Propane-Oxidizing Bacteria in the Presence of Hydrocarbons of the Aliphatic Series

Designated substrate	Average oxygen uptake in liters/hr/1 mg dry weight of bacteria	
	Mycobact. rubrum var. propanicum (No. 26)	Mycobact. species (No. 28-3)
Propane	13.194	8925
Hexane	32.627	20.055
Heptane	-	5.820

TABLE 4. Growth of Propane-Oxidizing Bacteria in an Atmosphere of Propane After Previous Cultivation on Meat-Peptone Agar

Culture No.	Microorganism	Bacteria growth rate after remaining in MPA for		
		20 days	30 days	137 days
26	Mycobact. rubrum	+	0	0
28—1	Mycobact. carotenum	+	+	+
28—3	Mycobact. species	+	+	0
6—3	Ps. species	+	+	0
6	» pantotropha	++	+	0
8	То же	+++	+	0
25	Pseudobact. subluteum	+	0	0

Note: Symbols as in Table 2.

Eight strains of bacteria were isolated in a propane atomosphere. According to morphological, cultural, and physiological characteristics three strains of propane-oxidizing bacteria belonged to the genus Mycobacterium (Mycobacterium rubrum, M. carotenum, Mycobacterium species), four strains to Pseudomonas (P. caudatus P. pantotropha), two of which were designated as Pseudomonas species, and one strain was shown to be Pseudobacterium subluteum.

All bacteria isolated in propane were aerobic, forming a film on the surface of liquid mineral medium when cultivated in a propane atmosphere.

In addition to a study of the morphological characteristics and species composition of the isolated bacterial cultures, special attention was devoted to the problem of their utilization of paraffin hydrocarbons as a source of carbon. Experiments were carried out with chemically pure methane, ethane, propane, butane, pentane, hexane and heptane. Pure cultures of bacteria were introduced into liquid mineral media and were cultivated in a mixture of hydrocarbons and air. Results of these experiments are presented in Table 2.

Examination of Table 2 shows that hydrocarbon-oxidizing bacteria isolated from waters and cores of gas locations in the Azov-Kuban depression behave differently with respect to the paraffin hydrocarbons.

Three basic groups may be designated according to their ability to oxidize aliphatic hydrocarbons. One of the above-mentioned groups, in which the representatives of the genera Mycobacterium and Pseudomonas are located, are capable of oxidizing only one hydrocarbon (propane or methane) out of four gaseous hydrocarbons. Mycobacterium rubrum and other species, Bacterium methanicum are also able to oxidaze the liquid hydrocarbons, hexans, hexane and heptane, in addition to propane and methane.

Another group of microorganisms differs in that its members can oxidize hydrocarbons from ethane and can utilize further all higher homologs. The rate of oxidation of hydrocarbons is approximately the same in all species of this group of bacteria. The cultures Pseudomonas caudatus (13), Pseudomonas species (6-3) and Pseudobacterium subluteum (No. 25) belong here.

The third group of bacteria, including Pseudomonas pantotropha, is characterized by ability to oxidize methane and to utilize further all higher homologs of this series. However, it must be noted that these species oxidize the heavier hydrocarbons more intensively—dark propane and butane among the gaseous hydrocarbons, pentane and hexane in the volatile hydrocarbons.

The rate of oxidation of hydrocarbons by Mycobacterium rubrum and related species was also determined by oxygen consumption — the manometric method in a Warburg apparatus. First the cultures were grown on a solid mineral medium in the presence of propane for 1-2 weeks. Gaseous and liquid hydrocarbons were used as oxidative substrates in these experiments. The amount of oxygen absorbed was related to the dry weight of the bacterial cells in 2 ml of suspension. The amount of oxygen required for endogenous respiration was calculated from the total oxygen utilized for the oxidation of each of the compounds.

The analytical data are presented in Table 3.

It can be seen from Table 3 that the highest rate of oxygen uptake occurred in the presence of hexane. The rate of oxygen utilization in the presence of propane was less rapid, and even less in heptane.

It has been stated in the literature a number of times that methane-oxidizing bacteria lose their ability to oxidize hydrocarbons after cultivation on meat media. In this connection we carried out a number of experiments with the cultivation of propane-oxidizing bacteria. The

indicated species were periodically transferred to fresh meat-peptone agar. At certain intervals, they were transferred from the meat-peptone agar to liquid mineral media and placed in an atmosphere of propane and air.

Results of the investigation presented in Table 4 indicate that cultures of propane-oxidizing bacteria gradually lose their ability to oxidize propane after growth on meat peptone agar.

SUMMARY

1. Bacteria utilizing gaseous hydrocarbons and inhabiting the waters of the gas-bearing regions of the Azov-Kuban depression, belong to the genera Pseudomonas, Mycobacterium, and Pseudobacterium.

2. Representatives of the genus Pseudomonas are represented by various species, which behave differently with respect to aliphatic hydrocarbons. One of them is strictly specific for methane, the others oxidize methane and all higher homologs of this series. Some species begin oxidation of hydrocarbons with ethane or propane and are not able to grow on the lighter hydrocarbons.

3. Representatives of the genera Mycobacterium and Pseudobacterium grow better in an atmosphere of the heavy gaseous hydrocarbons. Some species of the genus Mycobacterium are specific with respect to propane.

4. Microorganisms of the genera Mycobacterium, Pseudomonas and Pseudobacterium utilizing gaseous hydrocarbons as a sole source of carbon gradually lose their ability to oxidize hydrocarbons after growth on a complex organic medium (meat-peptone agar).

5. Loss of the oxidative powers of bacteria after growth on media containing organic substances is an indication of the specificity of the hydrocarbon-oxidizing bacteria.

LITERATURE CITED

1. G. A. Mogilevskii. 1958. Study of the Nature of Gas Bacterial Anomalies by Means of Borings and Gas Core Samplings [in Russian] 20th International Geological Congress. Data on Petroleum Geology. Vol. 1: Reports and Data on Russian Geology.
2. G. A. Mogilevskii. 1958. Coll.: Abstracts of Scientific Projects Completed in 1957.
3. O. P. Samoilova and Yu. N. Lisitsyna. 1958. Col.: Abstracts of Scientific Projects Completed in 1957.
4. W. E. Hutton and E. I. ZoBell.1949. J. Bact. 58. No. 4463.
5. E. Münz. 1915. Zur Physiologie der Methanbakterien. Dis., Schöneberg, Berlin.
6. N. L. Söhngen. 1913. Ztbl. Bakt., Abt. 11, 37, 595.
7. R.I. Strawinskii. 1955. Bact. Proc., p. 27 (cited by Davis, 1956).

DYNAMICS OF THE BACTERIAL MICROFLORA GROWTH IN CHLORELLA CULTURES

M. G. Vladimirova

K. A. Timiryazev Institute of Plant Physiology, Academy of Sciences, USSR
Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 431-435, May-June, 1961
Original article submitted February 5, 1960

The problem of the interrelationship between algae and bacteria has been of constant interest to investigators. Until the present, investigations have been concerned generally with the relationship between the numbers of phytoplankton and bacteria in ponds and with investigation of the role of phytoplankton in the self-purification of water.

A majority of investigators working in this field have noted the bactericidal properties both of fresh and sea water when living phytoplankton are present (Krasil'nikov, 1938; Razumov, 1948; Guseva, 1951; Alfimov, 1959; Lanskaya, 1960). An inhibitory action on bacterial growth has been shown by actively photosynthetic algae (Ostapenya, Levina, Kokina, 1957; Vinberg and Sivko, 1956). As most of the observations have shown, growth of algae almost always leads to an inhibition of growth of coliform bacteria, dysentery, and Staphylococcus aureus. Razumov (1948), Ruban (1959) and other authors noted an inverse relationship between the number of phytoplankton and the number of bacteria during the rapid growth phase of the algae while Guseva, 1951, and Vinberg and Sivko (1956) observed a passive relationship between saprophytic microflora and algal growth. The interrelationship existing between algae and bacteria is often looked on as antagonistic, not only because of antibiotic-like substances isolated from algae (Pratt, 1940; Levina, 1960; Gasilina, 1960), or changes in the pH of the medium caused by algae (Ostapenya, Levina, Kokina, 1957), but as a competition for nutrients between the algae and the bacteria (Guseva, 1951).

Lately, much more attention has been turned to the problem of mass production of algae (Gaevskaya, 1959; Vinberg, 1957; Tamiya, 1957; Myers, 1953 and others). However, data regarding the interrelationship developing between algae and bacteria during mass production of algae are still not sufficient.

The object of the present investigation was to trace bacterial development during the cultivation of algae and the production of a large number of algal cells in a medium in comparatively large closed vessels under conditions of minimum sterility.

A culture of Chlorella pyrenoidosa Pringh. was the experimental organism; it was grown on Tamiya's medium (Tamiya et al. 1953), through which air enriched with carbon dioxide was blown, in glass vessels or chambers of organic glass, volume 1.5-3.0 liters.

Preliminary sterilization of the vessels with 5% phenol or alcohol followed by careful rinsing with boiling water, the preparation of the medium from thoroughly boiled water, and the introduction of air through a sterile cotton filter completely prevented contamination of the cultures with protozoa, and in significant measure prevented contamination with fungi and bacteria. Some bacteria, nevertheless, got into the algae during the process of setting up the experiment. Tests showed that bacteria were usually introduced with the medium in the incubation flasks. Thus, if there were $21.5 \cdot 10^6$ or $3.9 \cdot 10^6$ bacterial cells per ml in an individual carboy during preparation, after setting up the experiment and mixing the suspension, the bacterial count was $30 \cdot 10^6$ or $4.5 \cdot 10^6$/ml, respectively, within half an hour.

The bacterial count was calculated by inoculation on Petri dishes with meat-peptone agar. Colony counts were carried out after 48-hr incubation at 37 C. The algal cell count was determined in Thom chambers.

Results showed that the growth both of algae and bacteria occurred for the first several days after introduction of a suspension of algae into the medium of the incubation flask, and that changes occurred in the progress of the growth of the culture only after a certain period in the algal growth cycle: the number of algae continued to increase and the number of bacteria began to decrease. At the end of the experiment in most cases the bacterial count was lower than at the beginning of the experiment. Examples of such observations are presented in Fig. 1. Results of an experiment are presented in Fig. 2 in which bacterial growth was inhibited during the first days of algal growth. Thus, inhibition of bacterial microflora occurs when they are cultivated in the presence of a considerable number of cells of Chlorella pyrenoidosa just as in the case of Chlorella vulgaris growth (Pratt, 1940) or other algae. It should be noted that

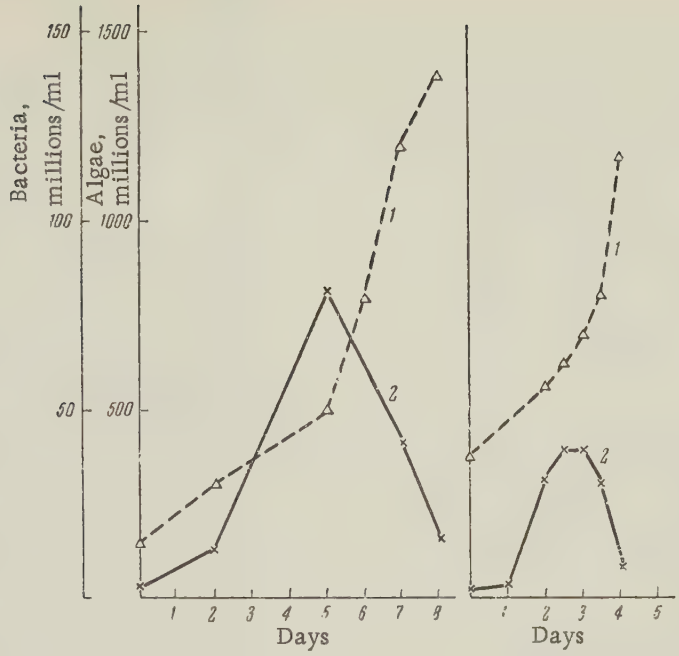


Fig. 1. Growth of bacteria in a culture of the alga Chlorella pyrenoidosa. 1) Algae; 2) bacteria.

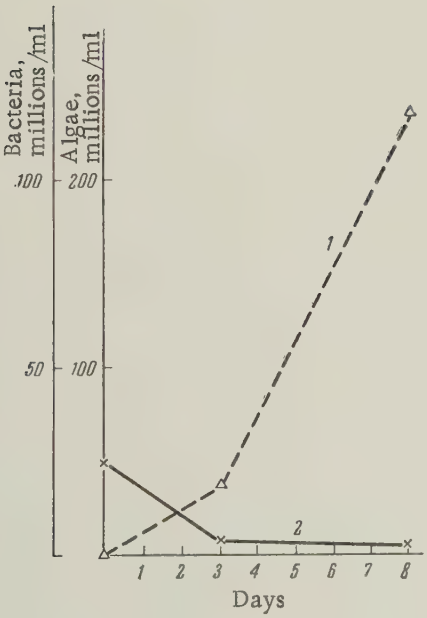


Fig. 2. Bacterial growth in a culture of the alga Chlorella pyrenoidosa. Bacterial growth was inhibited in the first days after development of the algae. 1) Algae; 2) bacteria.

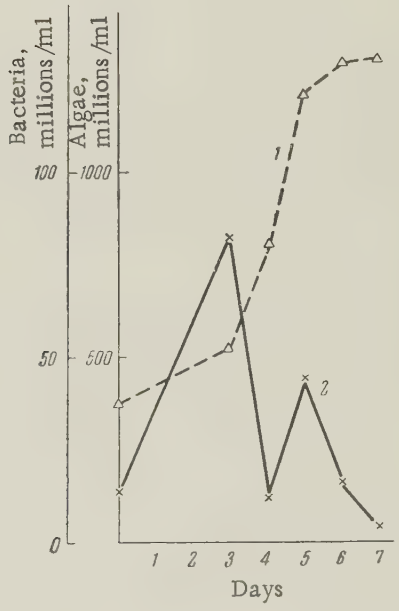


Fig. 3. Shift in bacterial forms in a culture of the alga Chlorella pyrenoidosa. 1) Algae; 2) bacteria.

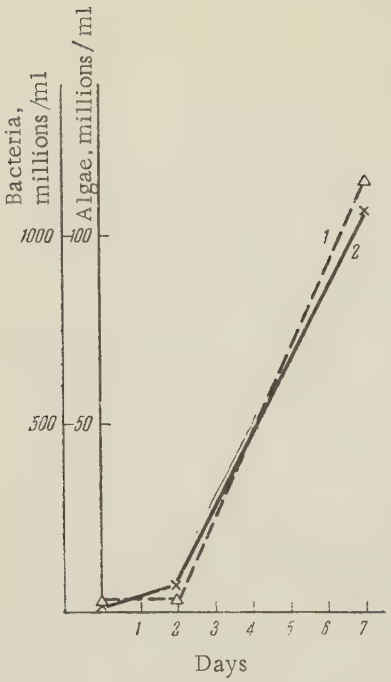


Fig. 4. Bacterial growth in algal cultures at a ratio of bacterial and algal cell counts unfavorable to Chlorella growth at inoculation. 1) Algae; 2) bacteria.

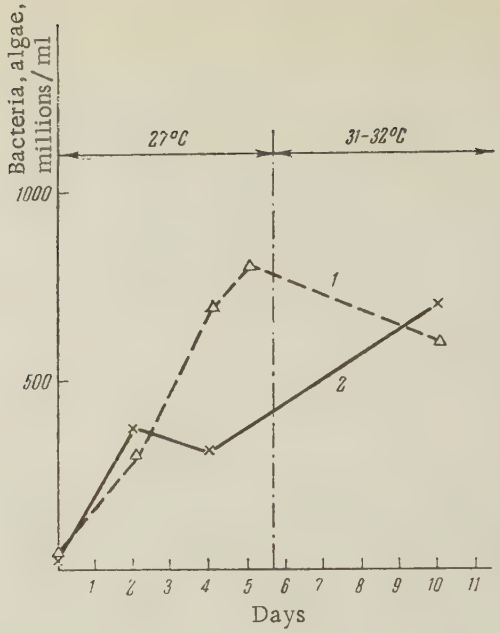


Fig. 5. Effect of temperature on bacterial growth in an algal culture. 1) Algae; 2) bacteria.

the dominant form of contaminating bacteria in all our experiments was always fluorescent, small motile rods. The bacteria were isolated in pure culture, put through a variegated series of tests, and by morphological and biochemical characteristies were shown to be closely related to Pseudomonas oleovorans (Bergey's Manual, 1948). However, in distinction from P. oleovorans, the pseudomonas growing in our cultures formed acid on glucose, alkalized milk and hydrolyzed starch only to a slight extent.

This form of bacteria continued to grow in the culture of algae even after the remaining microflora were already suppressed. In Fig. 3 data from an experiment are presented, in which a new outbreak of bacterial growth suddenly took place after the initial inhibition of growth. In this case a shift in bacterial forms was observed: in addition to the primary diverse forms of microflora in the culture of algae, bacteria of the Pseudomonas oleovorans type began to develop.

However, those bacteria that were more adapted to growth in the chlorella cultures also were inhibited on continued algal growth.

The process of self-purification from bacteria of algal cultures and its rate must obviously depend on a number of factors. Thus, in the experiment shown in Fig. 4, one of the determining factors was shown to be the initial ratio between the number of bacteria and algae. When this experiment was set up the bacterial cell count and the algal cell count were 11.3 and 0.08 million/ml, respectively, that is, the number of bacteria exceeded the number of algae by approximately 100 times. As a result, the initial rapid multiplication of bacteria markedly inhibited the development of algae, so that visible growth of Chlorella began only after 5-6 days of incubation, while rapid growth of the algae was usually within 1-2 days. In another experiment (Fig. 5), temperature was shown to be a decisive factor. Normal growth of this strain of Chlorella pyrenoidosa occurs at 25-27 C; at 30-32 C growth of the algae is retarded, and with long exposure to this temperature the algae generally die; on the other hand, this temperature is more favorable for the growth of bacteria. Increasing the temperature of the suspension from 27 C to 31-32 C led to inhibited growth of the algae and that in itself removed the suppression of bacterial growth begun by Chlorella in the process of normal growth. Thus, the ratio between the number of algae and bacteria in the Chlorella culture was a determining factor favorable to the growth of bacteria as well as to the active state of the algae.

SUMMARY

Growth of the alga Chlorella pyrenoidosa Pringh. suppresses the growth of bacterial microflora.

A fluorescent bacterium of the genus Psedomonas was best adapted to growth in the same conditions as the algae when the Chlorella cultures were contaminated

with bacteria. Growth of this species of bacteria was also inhibited during the process of normal growth of the algal cultures.

The initial ratio of the bacterial and algal cell count, as well as the incubation temperature, had an effect on the quantitative ratio of bacteria to algae existing during cultivation of algae.

LITERATURE CITED

1. N. N. Alfimov, 1959. Botan. Zh. 44, No. 6.
2. G. G. Vinberg and T. N. Sivko, 1956. Tr. Vses. Gidrobiol. Obsh. 7.
3. G. G. Vinberg, 1957. Usp. Sovr. Biol. 21, No. 3.
4. N. C. Gaevskaya, 1959. Vopr. Ikhtiol., No. 12.
5. M. M. Gasilina, 1960. Tr. Nauch. Soveshch. Feb. 8-13, 1960, Minsk.
6. K. A. Guseva, 1951. Tr. Problemn. Temat. Soveshch. ZIN, No. 1.
7. N. A. Krasil'nikov,1938. Mikrobiologiya 7, No. 3.
8. L. A. Lanskaya, 1960. Tr. Nauch. Soveshch. Feb. 8-13, 1960, Minsk.
9. R. I. Levina, 1961. In the collection, Purification of Sewage in Biological Ponds [in Russian] Izd. AN BSSR, Minsk, p. 136.
10. P. V. Ostapenya, R. I. Levina, and A. G. Kokina, 1957. Conference on the Use and Detoxication of Sewage in Agricultural Irrigated Fields [in Russian] Moscow.
11. A. S. Razumov , 1948. Problems of Sanitary Bacteriology [in Russian] Moscow.
12. E. L. Ruban, 1959. Tr. Vses. Gidrobiol. Obsh. 9.
13. Bergey's Manual of Determinative Bacteriology, 1948. 6-th ed.
14. J. Myers, 1953. Carnegie Inst. Wash. Publ. No. 600, 37.
15. R. Pratt, 1940. Amer. J. Bot. 27, 52.
16. H. Tamiya, E. Hase, K. Shibatc, A. Mituya, T. Iwamura, T. Nihei, and T. Sasa, 1953. Carnegie Inst. Wash. Publ. No. 600, 204.
17. H. Tamiya, 1957. Studies from the Tokugawa. Inst. VIII, No. 1.

SOME DIRECT EFFECTS OF IONIZING RADIATIONS ON THE YEAST CELL

K. Vas and I. Farkas

Budapest Central Scientific Research Institute of Food Industry
Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 436-440, May-June, 1961
Original article submitted May 10, 1960

The mechanism of action of radiation on cells constitutes one of the basic questions in radiobiology. This question is not only of theoretical significance, but is also of considerable practical interest from both the medical and industrial points of view (radiation sterilization of foods and drugs).

In part, the problems pertaining to mechanism of action of radiation are relegated to physical, chemical, and biochemical changes occurring immediately and independently in irradiated cell. The present work is devoted to these questions and is based on experiments carried out with two types of ionizing radiations: roentgen rays from a 250 kv machine and electron beam of Van de Graaff accelerator (1000 kv).

In his work with ionizing radiation Loofbourow (1947) found that amino acids and other compounds are released from the yeast cells as a result of action of ultraviolet rays. These results are similar to those noted by Gale and Taylor (1947) in the course of action of detergent on a cell, and by one of us (Vas, 1953) in a series of other interactions. Manifestation of this effect is exemplified by diffusion into the surrounding medium of the accumulated amino acids (and nucleic acids) from gram-positive yeast cells. This process is correlated with damage to cytoplasmic membrane and is shown by increased permeability. Similar release of phosphate compounds has been demonstrated by Hevesy and Zarahn (1946) and Meisel' (1955).

It should be pointed out that a greater part of these studies is devoted to action of low radiation doses, and to study of progeny of irradiated cells. Direct action of large radiation doses attracted little attention, except for the study by Meisel' et al. (1955, 1958).

METHODS

In this investigation a study was made of direct alterations in permeability of cytoplasmic membranes of baker's yeast (Saccharomyces cerevisiae) occurring immediately after exposure to large doses of radiation. Free reserve amino acids liberated from a cell were studied by chromatography on paper. Material for chromatographic analysis was prepared in the following manner. The yeast suspension was centrifuged and 1-2 ml of supernatant fluid was removed. The liquid was evaporated to 0.1 to 0.2 of the original volume, and a definite quantity of this concentration was placed on Watman #1 paper, which was marked by rhomboid openings at the start. Descending chromatography was used, using as solvent a mixture of butanol, glacial acetic acid, and water in 70:15:15 ratio. The time of reaction was 10-20 hr. A 0.1% solution of ninhydrin in concentrated aqueous butanol was used as developer. The development was carried out at 100 C for 5 min. A study was also made of the dehydrogenating activity of a 20% suspension of irradiated yeast (approximately 2×10^9 cells/ml). In the latter case, use was made of the reaction of restoring triphenyltetrazoliumchloride in nutrient media at pH 7.2 after 18 hr incubation at 28 C (Barnes, 1956). For roentgen rays (200 kv), a dose of 0.8 kr/min was used, and with the Van de Graaff generator, a radiation dose of 400-800 krep/min. Ferrosulfate and cerium sulfate dosimetry was used (Weiss, 1952). For irradiation, 20-25 ml of yeast suspension, washed and centrifuged 3 times in distilled water, was placed in a Petri dish 70 mm in diameter (thickness of yeast suspension layer was 3-4 mm). For irradiation with electrons, 0.4 ml of suspension was placed in an aluminum container 20 mm in diameter and 20 mm high (thickness of layer of suspension was 1.3 mm). Aluminum containers were coated inside with a layer of lacquer (CIBA Aratdit Lachharz 985ECZ).

In order to give a clearer picture of the results obtained in chromatographic analysis, the data are presented by diagrams, on which spots are symbolized by circles, which diameters are proportional to the visually determined size and intensity of spots on chromatograms. Position of circles on the diagram corresponds to R_f of spots. In this manner it is possible to compare easily the results obtained on chromatographs started at different times and with different amounts of material.

RESULTS

Comparison of the two types of radiation showed that, using a similar dose (300 kr or 300 krep), diffusion of amino acids is eight times more extensive after

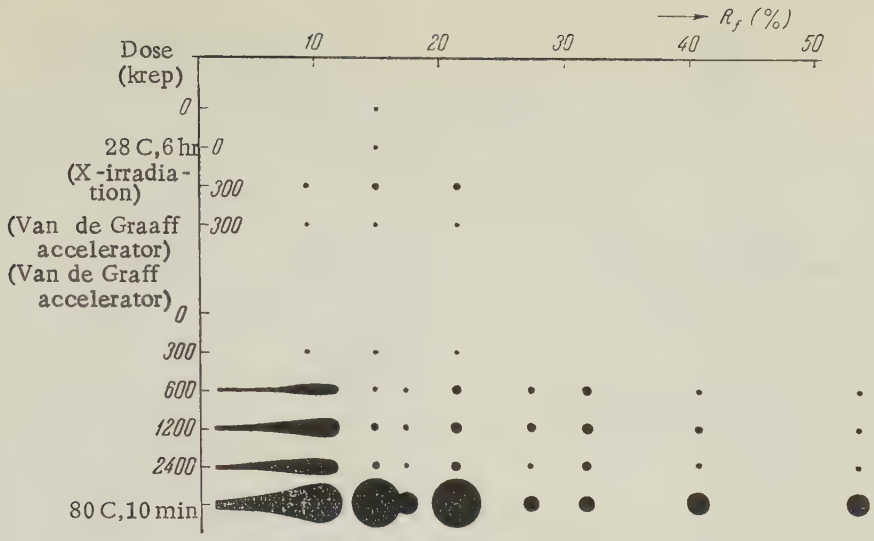


Fig. 1. Explanation in text.

x-irradiation than after exposure to electrons (upper part of Fig. 1).

Most experiments were carried out with the Van de Graaff generator, inasmuch as it allows for simpler procedure in exposure to radiation and shorter time for irradiation, which allows carrying out a large number of parallel experiments using high radiation dose. Results of one such experiment are presented in Fig. 1.

Diagrams of chromatograms illustrating effects of different doses (from 300 to 2400 krep) of radiation on the amount of free extracellular amino acids are shown in the lower part of this figure. Just as in the upper part of this figure, it can be seen here that the liberated amino acids, as determined by analytical procedures, are first detected at 300 krep, the process intensifies at 600 krep and attains maximum at 1200 krep, and continues essentially unchanged up to 2400 krep dose. For comparison there is shown in the same figure a chromatographic diagram of a sample of amino acids prepared in the following manner: 20% yeast suspension in distilled water was heated for 10 min at 80C. A sample for analysis was removed from supernatant fluid after centrifugation. From these results it is possible to determine the total amount of non-bound intracellular amino acids, and to make an interesting comparison of radiation and heating effects. In this manner, it is seen that there is quite incomplete penetration of irradiated cytoplasmic membrane. It can be also pointed out that not all amino acids leave the cell in the same state. If we should consider the total free amino acids represented by extracellular amount obtained after heating, and consider this equal to 100%, then after exposure to 1200 krep we obtain the following relative amounts of free amino acids (Fig. 2).

In this manner it is seen that on the one hand there is partial damage to cytoplasmic membrane, and on the other hand it is selective in nature: the membrane becomes more permeable to some amino acids, and less permeable to other amino acids. In another experiment we studied the total and relative amounts of α- amino nitrogen (measured colorimetrically in the supernatant fluid, using ninhydrin, Vas, 1953) liberated from the cell during irradiation with different doses of electrons (curve 2 in Fig. 3). It has been established that diffusion begins at 300 krep. Increase in dose is exemplified in sharp increase in quantity of freed nitrogen, reaching a maximum at 1200 krep.

In the subsequent series of experiments we studied alterations in dehydrogenating activity, measured by restoration of triphenyltetrazolium (TTZ). As can be seen from curve 1 (Fig. 3), the reducing activity of a yeast suspension is markedly lowered even at 75 krep, reaching a minimum at 150-225 krep. Beginning with 300 krep reducing activity increases. Reducing activity is first manifested at 500 krep. From 600 to 1200 krep there is a gradual increase in reducing activity. There are no changes from 1200-2400 krep, after which there is a marked reduction in activity during the 2400-4800 krep interval.

Comparative data of alteration of reducing activity of yeast cells are presented in the upper part of Fig. 3, and diffusion of amino acids in the lower portion of the figure. It should be pointed out at this time that according to our data the number of living yeast cells decreases by more than 99% after exposure to 150 kr.

By comparing the indicated values, one can arrive at the following explanation of the observed phenomena. Doses above 150 krep "break down" most viable cells, or to be more exact, lead to their loss of ability to mutiply and form colonies. This is probably related to certain damage to cellular fermentative system, which is manifested in lowering of dehydrogenating activity. The lat-

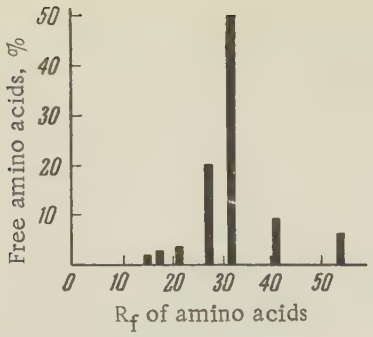


Fig. 2. Explanation in text. Abscissa – R_f of amino acids; ordinate – free extracellular amino acids, in percent of the total cell amino acids.

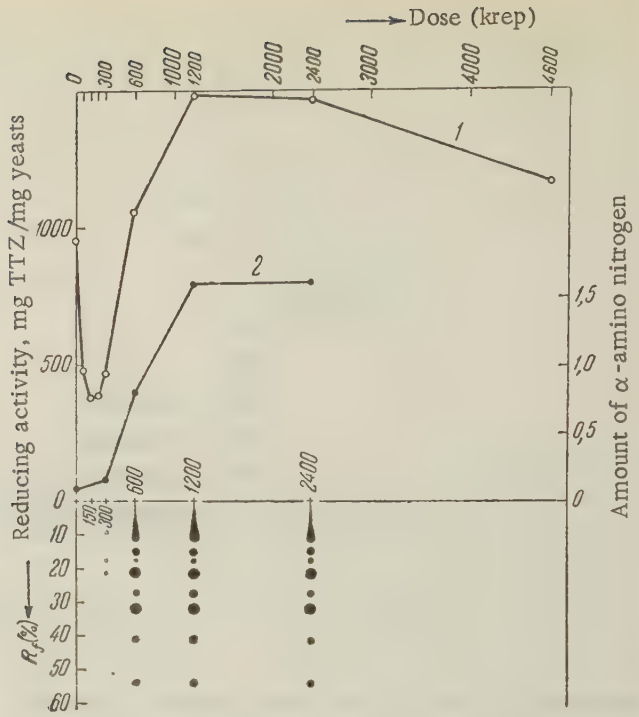


Fig. 3. For explanation see text. 1) Reducing activity of yeast; 2) diffusion of amino acids.

ter aspect was demonstrated by Meisel' et al. (1958) in utilization of neutral red and janus green. Similar to our data, significant damage to cytoplasmic membranes begins with doses above 300 krep, and is expressed particularly clearly at 600 krep. This damage is indicated not only in liberation of reserve amino acids from a cell, but also by facilitating diffusion of triphenyltetrazolium into the cell, in restoring it to formazan. One can assume that marked increase in dehydrogenating activity at doses of 600 krep may certainly be explained in this manner. On the other hand, permeability of membrane to triphenyltetrazolium is not increased even after exposure to 1200 krep. At high doses the enzyme-inactivating activity of radiation may prevail, masking, for example at 2400 krep, possible increase in permeability to the chemical. One may assume that this is the reason for absence of increased reducing activity of the suspension. According to the literature, radiation inactivation of enzymes may be lowered at 4800 krep; the latter may serve to explain the observed reduction in formation of formazan.

SUMMARY

This investigation was concerned with the direct effects of large doses of ionizing radiation (roentgen rays and electrons) on the permeability of the cell membrane and dehydrogenating activity of Saccharomyces cerevisiae. The permeability of the cellular membrane was evaluated from the amount of released free amino acids after irradiation (determined by paper chromatography). Dehydrogenase activity was determined using triphenyltetrazolium. It has been established that release of amino acids from cells is first detected at 280 krep. Increasing the dose to 1100 krep leads to a corresponding increase in diffusion. At equal doses, x-rays are more effective than electron beams. The dehydrogenase activity of yeast suspensions shows a considerable decrease with increasing doses up to about 140-210 krep. Further increase in the dose, up to about 1100-2200 krep, strongly enhances the formation of triphenylformazan; above this level at 4500 krep, a decrease in dehydrogenase activity may again be noticed.

LITERATURE CITED

1. M. N. Meisel', 1955. International Conference on the Peaceful Uses of Atomic Energy 8: 690.
2. M. N. Meisel', et al., 1958. The Second International Conference of the Uses of Atomic Energy 15: 2320.
3. E. M. Barnes, 1956. J. Gen. Microbiol. 14: 57.
4. E. F. Gale and E. S. Taylor, 1947. J. Gen. Microbiol. 1: 77.
5. G. Hevesy and K. Zerahan, 1946. Acta Radiol. 27: 316.
6. J. R. Loofbourow, 1947. Biochem. J. 41: 119.
7. K. Vas, 1953. Agrokémia és Talajtan 2, 1.
8. J. Weiss, 1952. Nucleonics 10, 28.

POSSIBILITY OF ULTRASONIC PRODUCTION OF NON-LYSOGENIC VARIANTS FROM LYSOGENIC ACTINOMYCES CULTURES

Ya. I. Rautenshtein, N. P. Fadeeva, and I. E. Él'piner

Institute of Microbiology and Institute of Biophysics, Academy of Sciences, USSR

Translated from Mikrobiologiya, Vol. 30, No. 3, pp. 441-446, May-June, 1961
Original article submitted January 20, 1961

The presence of indicator cultures, i.e., cultures sensitive to phage present in a given lysogenic culture, represents an essential condition for studying the phenomenon of lysogeny.

Usually indicator cultures are observed in cross-testing for lysogeny a number of strains of the same species. In this manner, it was possible to establish the lysogenic nature and to select indicator strains in a series of Actinomyces: Actinomyces levoris (Rautenshtein, 1957), Actinomyces olivaceus (Khavina and Rautenshtein, 1958), Actinomyces lavendulae (Rautenshtein and Khavina, 1961), and Actinomyces of different species (Welsch, 1959).

An indicator culture may also be obtained from lysogenic culture when the latter forms non-lysogenic variants. This phenomenon has been described for Bacillus megaterium (Lwoff, 1953; Stolp, 1957, 1958; Rautenshtein, Misyurova, Krongauz and Filatova, 1960).

Only meager data are available regarding the possibility of loss of lysogenic property. Bradley (1959), in his comments regarding Gold's report (1959) pertaining to the influence of the nature and composition of medium on activity of actinophage of Streptomyces griseus, notes that he observed only once a loss of lysogenic property in Actinomyces, without describing in which manner he was able to obtain the non-lysogenic variant.

The purpose of this investigation was the determination of a possibility for obtaining non-lysogenic variants by subjecting lysogenic cultures to ultrasonics. In this we assumed that among the broken fragments of mycelium, arising from its disruption by ultrasonics, there may be some that do not harbor prophage. If among the fragmented mycelium without prophage there will be viable cells, then these should give rise to a non-lysogenic culture which should serve as an indicator strain in relation to the original lysogenic culture.

METHODS

Two equally active lysogenic cultures with their indicator strains were used in the experiments: A. olivaceus strain #132 (lysogenic) and its indicator culture A. olivaceus strain #1225, and A. venezuelae strain #10712 (lysogenic) and its indicator culture A. venezuelae strain #8231.

In addition, there was utilized one variant of A. erythreus strain #8594, designated by us as strain L_2, received from the Museum of Live Cultures of the All-Union Scientific Research Institute of Antibiotics (VNIIA)*

We chose this strain for the following reasons. It has been shown by Retinskaya and Rautenshtein (1960) that A. erythreus and all its variants are lysogenic. In inoculating these cultures over the entire agar surface, there appears either a larger or a smaller number of plaques. Strain L_2 differs from other variants of culture #8594 in that there are many plaques when it is used as inoculum. These authors were unable to obtain actinophage from a culture of A. erythreus and from its variants because of lack of an indicator culture. The actinophages obtained by the authors from placing filtrates of different agents on surface growth of A. erythreus were considered by them to be virulent variants of symbiotic phage of a given culture, capable of lysing host culture.

Experimental cultures were grown on a shaker in flasks with fish medium at 27 C. The fish medium consisted of 2 % fish extract and 2% glucose in tap water. After growing for 24 hr, 2 ml of the culture with developed mycelium was transferred into test tubes, which were then exposed to ultrasonic sound. Experiments were carried out with mycelium which had been washed several times in physiological solution and centrifuged. In these experiments mycelium suspended in physiological solution was subjected to ultrasonic sound.

Quartz piezo-electric generator served as source of ultrasonics having a frequency of vibration about 650 kc and intensity of ultrasonic waves about 20 w/cm^2. Test tubes subjected to ultrasonics were continuously cooled.

* This strain was described by M. M. Meksina in the Bulletin of VNIIA #5/33, 1957. Authors acknowledge their appreciation to M. M. Meksina for making available this culture.

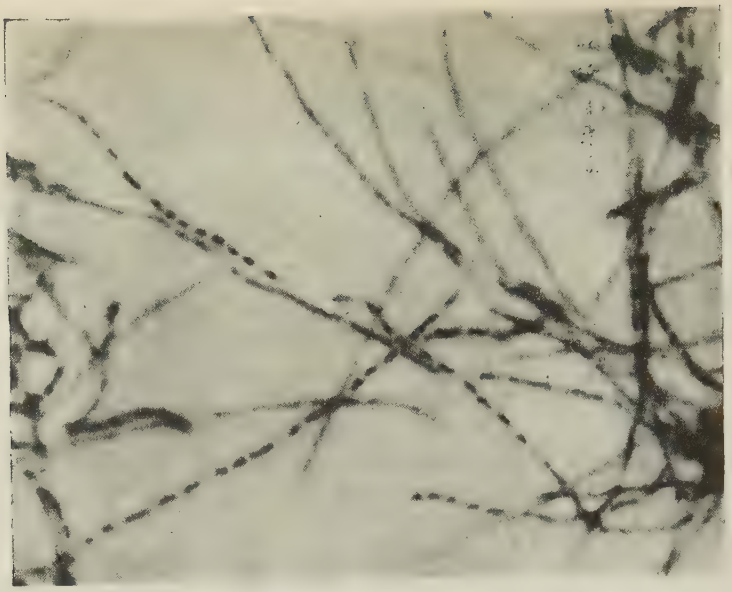


Fig. 1. Mycelium of a 24-hr culture of A. venezuelae strain 10712. Carnoy's fixative, stained with methylene blue, ×1530.

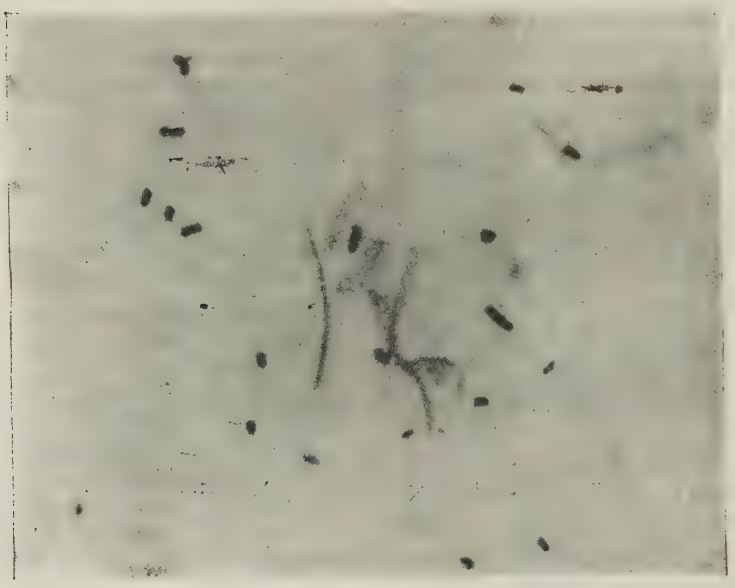


Fig. 2. Mycelium of a 24-hr culture of A. venezuelae strain 10712 after sonic treatment for 20 min. Carnoy's fixative, stained with methylene blue, × 1530.

The time of exposure to ultrasonics varied with the nature of the culture. A. olivaceus and A. erytheus, forming smaller microcolonies, were exposed from 30 to 60 min and A. venezuelae from 10 to 20 min.

After treatment the culture, containing mycelial fragments, was diluted with broth to yield, after seeding in Petri dishes with corn-peptone agar medium I, up to 200 colonies. † Plates were held at 27 C for 3-5 days and then covered with a mixture of 2 ml corn-peptone agar cooled to 50 C and containing 1.5% agar in 2 ml of the corresponding 24-hr-old culture. The plates were again incubated at 27 C and were examined after 24-48 hr.

Preliminary study of experimental lysogenic cultures of A. olivaceus and A. venezuelae showed that after covering their 3-5 day old colonies grown on medium I with agar containing the corresponding indicator culture, a clear zone of absence of growth of indicator culture is

† Composition of medium I (in %): glucose, 1.0; peptone, 0.5; corn extract, 0.5; NaCl, 0.5; $CaCO_3$, 0.5; agar, 1.5; pH, 7.0.

TABLE 1. Lysogenic Cultures of A. olivaceus and A. venezuelae Developed from Mycelial Fragments Obtained by Exposure to Ultrasonics

Experimental lysogenic culture	Indicator culture	Colonies developing after sonic-treated mycelium inoculation	Number of lysogenic colonies
Act. olivaceus № 132	Act. olivaceus № 1255	9229	9229
Act. venezuelae № 10712	Act. venezuelae № 8231	20 545	20 545

formed around each lysogenic colony. This sterile zone appears as a result of lysis of indicator culture by actinophage released by lysogenic culture.

Serial transmission and isolation from this zone of actinophage, which could multiply on indicator culture, served as proof that the clear zone of no growth of indicator culture is brought about by its lysis of actinophage.

Experimental Results with Cultures of A. olivaceus and A. venezuelae

A. olivaceus strain 132 forms microcolonies with dense central part from which originate straight hyphae, after growing 24 hr on a shaker in fish broth. Mycelial protoplasm is basophilic at this time. As a result of 30-60 min of sonictreatment mycelium breaks up into minute fragments, with less basophilic cytoplasm.

Cultivation of mycelial fragment on medium I led to development of colonies which could not be differentiated in appearance from colonies from normal mycelium.

A. venezuelae under identical conditions of growth forms smaller microcolonies, with short, peripheral hyphae. Protoplasm of 24 hr mycelial growth is well differentiated. As a result of 10-20 min of sonictreatment, the mycelium breaks down into small fragments (Figs. 1 and 2). In growing these fragments on medium I, the developing colonies also did not differ morphologically from colonies formed by normal culture.

The first series of experiments was carried out with sonictreated mycelial preparations of both of the above indicated cultures; the mycelium was not washed prior to treatment. The results of these experiments are presented in Table 1.

It is evident from the data presented in Table 1 that all cultures obtained from mycelial fragments of a lysogenic culture formed by sonictreatment of the original culture were also lysogenic.

Petri dishes with colonies of A. venezuelae (Figs. 3 and 4) and A. olivaceus (Fig. 5) formed from mycelial fragments and covered with semi-solid agar with indicator cultures are shown in Figs. 3, 4, and 5.

The figures show that around each colony of actinomycetes developed from mycelial fragments there clearly is no growth of the indicator culture. These zones of no growth were brought about by phage in the lysogenic culture. This contention was confirmed by serial passage experiments. In certain cases presence of a clearly expressed zone of lysis around the colony was doubtful. All cultures of such colonies were investigated for lysogenic properties. In no case was there absence of lysogeny among these cultures.

Therefore, we were unable to isolate non-lysogenic variants from lysogenic A. olivaceus strain #132 and A. venezuela strain #10712 by applying the experimental procedures outlined above. Both of the above lysogenic cultures freely release intracellular phage when grown on a shaker in fish broth. In this manner, the titer of phage in culture fluid reaches 10^3-10^4. Therefore, it would be reasonable to expect that, should prophage be absent from mycelial fragments resulting from sonic treatment (i.e., from fragments which should produce non-lysogenic phage sensitive variants), then such variants will be lysed by phage contained in culture fluid, which will be deposited on nutrient surface together with the dispersed fragments.

To remove phage found in culture medium, mycelium of experimental cultures was repeatedly washed with sterile physiological saline and subsequently subjected to centrifugation, until complete removal of free phage. Washed mycelium suspended in physiological saline and without extracellular phage was treated with sound.

Experiments with dispersion of sound-treated mycelium without free phage were carried out on cultures of A. venezuela #10712.

Actinophage of this culture is quite sensitive to citrates. By addition of 0.25% sodium citrate to medium I, the actinophage was prevented from lysing the sensitive indicator culture A. venezuela #8231.

In this connection, additional experiments were designed with lysogenic culture A. lavendulae grown in sodium citrate prior to sonictreatment, as well as with spreading mycelial fragments after treatment, on medium containing citrate.

Experimental results with mycelium washed with physiological saline until complete removal of phage,

as well as results of growing mycelium and its fragments on citrated media, are presented in Table 2.

From the data presented in Table 2 it can be seen that colonies of lysogenic culture developed from all mycelial fragments of lysogenic culture A. venezuela #10712 capable of growth, regardless of whether they were obtained by sonictreatment of mycelium washed prior to exposure to completely remove phage, or from mycelium grown on citrated medium. Consequently, prophage was contained in each mycelial fragment capable of development.

Experiments in Isolation of Non-Lysogenic Variant from a Culture of A. erythreus

As indicated above, no indicator strain was available for experimental culture A. erythreus strain L_2. The search for the latter was made in the following manner. The culture was grown on a shaker in fish broth at 27 C for 48 hr. Sonic treatment was for 30-60 min. Colonies developing after inoculation of sonic treated material on medium I were transferred to test tubes. Each culture isolated from a colony was tested for sensitivity to actinophage, which was assumed to be present in culture fluid of L_2 grown on a shaker in flasks with fish broth at 27 C. To this end, after 24 to 48 hr of growth the culture fluid was centrifuged, and supernatant was placed on freshly seeded inocula of all individual isolates of strain L_2. The plates were examined daily for 4 days.

In all, 500 cultures of A. erythreus obtained from isolated colonies were tested. The greater majority of cultures grown from individual fragments after sonic treatment did not differ from the parent culture L_2 in the morphology of their colonies.

In only one experiment, 3 of 100 colonies developed from dispersed mycelium sonic treated for 60 min differed sharply from the parent culture in pigmentation. The three cultures formed dark cherry-colored pigment, diffusing into the medium. They differed also in poor spore formation. These cultures, similar to the parent L_2, were sensitive to actinophage specific for A. erythreus and produced erythromycin. Meksina‡ observed the appearance of variants of this type in the course of normal cultivation of A. erythreus.

Not one of 500 cultures of A. erythreus isolated after sonic treatment was sensitive to actinophage in lysogenic parent L_2 culture. Consequently, experiments designed to obtain non-lysogenic variants from lysogenic A. erythreus proved also negative.

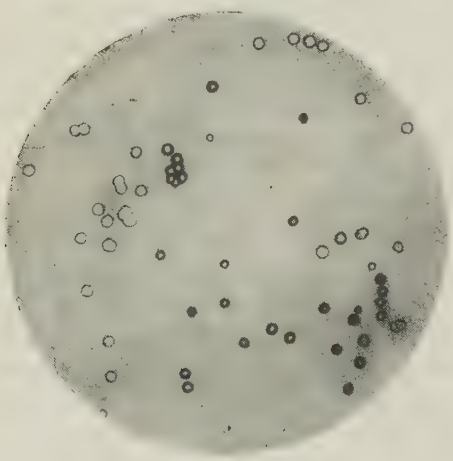


Fig. 3. Colonies of A. venezuelae strain 10712 from sonic treated mycelial fragments. 48 hr. Overlay A. venezuelae strain 8231.

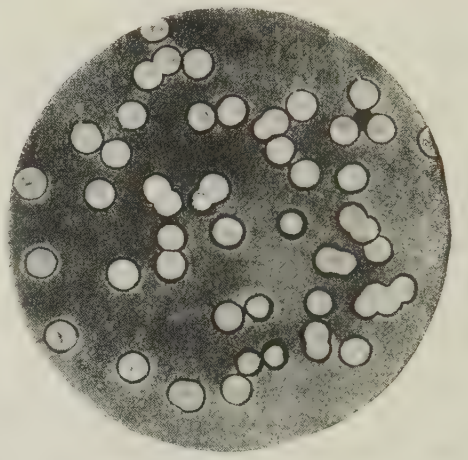


Fig. 4. Colonies of A. venezuelae strain 10712 from sonic treated mycelial fragments. Five days. Overlay of A. venezuelae strain 8231.

Fig. 5. Colonies of A. olivaceus strain 132 from sonic treated mycelial fragments. Five days. Overlay of A. olivaceus strain 1255.

‡ Bulletin of the All-Union Scientific Research Institu of Antibiotics [in Russian] (1957) No. 5, p. 33.

TABLE 2. Lysogenic Culture A. venezuelae strain #10712 Developing from Fragments of Washed Mycelium and on Medium with Sodium Citrate

Experimental material	Number of developed and studied colonies on medium I	Of these, number of lysogenic colonies	Number of developed and studied colonies on medium I +0.25% sodium citrate	Of these, number of lysogenic colonies
Spores of A. venezuelae strain #10712	4650	4650	3280	3280
Mycelium washed in physiological saline until complete removal of free phage, not sonic treated	3650	3650	3010	3010
Mycelial fragments washed in physiological saline until complete removal of free phage, then subjected to sonic treatment for 10 min	4000	4000	3460	3460
Mycelium grown in fish broth +0.25% sodium citrate and washed in physiological saline until complete removal of free phage	2800	2800	2675	2675
Mycelial fragments grown in fish broth +0.25% sodium citrate and washed until complete removal of free phage, then treated with sound waves for 10 min	2670	2670	3210	3210

However, negative results with the 3 tested cultures of actinomycetes do not form, under any conditions, a basis for contention of impossibility of obtaining experimental non-lysogenic variants from lysogenic cultures. Rather, we are convinced that it is possible to obtain these variants and consider it necessary to carry out additional investigations in this connection.

At the same time the following important conclusion appears from our results.

It is known that in lysogenic bacteria phage is present as prophage, which is related to the nuclear apparatus of the cell. In this stage phage is capable of multiplication, without cellular destruction, and after division of the latter is transferred to the daughter cells.

The developmental and multiplication processes are different in actinomycetes than in bacteria. In actinomycetes, individual mycelial fragments are capable of regeneration.

Our data show that lysogenic state is stable in actinomycetes, as it is in bacteria. From this one may conclude that in the course of mycelial growth of lysogenic cultures of actinomycetes, prophage multiplies along with development of nuclear apparatus, and that prophage together with nucleoids is distributed uniformly along the entire mycelium. Therefore, in breakup of mycelium by ultrasonics the greater majority of fragments containing nucleoids and capable of growth also contain prophage and give rise to lysogenic cultures.

SUMMARY

This investigation was concerned with the possibility of obtaining prophage-free variants from 3 lysogenic cultures of Actinomyces (A. olivaceus strain #132, A. venezuelae strain #10712 and A. erythreus strain #8594) which consequently became sensitive to actinophage of the original culture. Indicator strains were available for 2 cultures: A. olivaceus and A. venezuelae.

All experimental cultures were treated with high-frequency sound waves in order to establish the presence among the formed mycelial fragments of such specimens which were able to germinate, but at the same time did not contain prophage.

It was not possible to obtain variants that lost prophage in any of the experiments. Colonies of lysogenic culture developed from all, even minute, mycelial fragments, i.e., all these fragments contained prophage.

It will thus appear that in lysogenic Actinomyces cultures growth of the mycelium is accompanied by reproduction of the prophage, which is uniformly distributed throughout the length of the mycelium together with the nucleoids of the culture. Hence, it is present in minute fragments of the mycelium able to grow and secures the stability of the lysogenic status of the culture.

LITERATURE CITED

1. Ya. I. Rautenshtein. 1957. Mikrobiologiya 26 (5): 573.

2. Ya. I. Rautenshtein, N. G. Misyurova, E. A. Krongauz, and A. D. Filotova. 1960. Mikrobiologiya 29 (4): 571.
3. Ya. I. Rautenshtein and É. S. Khavina. 1961. "Actinophages of cultures of A. lavendulae." Izv. AN SSSR, No. 2, 289.
4. É. S. Khavina and Ya. I. Rautenshtein. 1958. Mikrobiologiya 27 (4): 442.
5. V. M. Retinskaya and Ya. I. Rautenshtein. 1960. Microbiologiya 29 (6): 849.
6. S. G. Bradley. 1959. Ann. N. Y. Acad. Sci. 81: 994.
7. A. Lwoff. 1953. Bact. Rev. 17, 269.
8. H. Stolp. 1957. Arch. Mikrobiol. 26; 55.
9. H. Stolp. 1958. Arch. Mikrobiol. 31; 262.
10. M. Welsch. 1959. Ann. N. Y. Acad. Sci. 81; 974.
11. W. Cold. 1959. Ann. N. Y. Acad. Sci. 81; 994.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

INFLUENCE OF SOME AMINO ACIDS ON THE GROWTH AND ACID PRODUCTION OF THE Aspergillus niger T_1 MUTANT

I. D. Kasatkina

Institute of Microbiology, Academy of Sciences, USSR
Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 447-452, May-June, 1961
Original article submitted November 10, 1960

Under the influence of ultraviolet or Roentgen irradiation, microorganisms often produce mutants with various disturbances of the enzyme systems connected with the synthesis of cellular material.

In some instances we encounter a complete loss of the enzyme systems responsible for individual biochemical synthetic reactions. Such is the case with microbial biochemical mutants which cannot synthesize amino acids, vitamins, purine or pyrimidine bases, etc. In other instances the organism incurs a retarded rate of some of the enzymatic reactions which lead to the synthesis of an essential metabolite. Such mutants are characterized by a slower growth on the usual nutrient media as compared to the original, unirradiated form. In both instances the mutant's delay in growth and development can be overcome by using more completely nutritive media which provide, in ready form, those substances whose synthesis within the organism has been disrupted or weakened. Imshenetskii, Solntseva and Kuranova (1959), using ultraviolet rays, obtained the Aspergillus niger mutant T_1 which formed more citric acid than the original industrial Aspergillus culture. The ultraviolet T_1 mutant showed a slower growth on sucrose – mineral medium, and yielded a biomass 25 – 30% lower than the original culture of this fungus. One would expect that growing the A. niger T_1 variant on a more completely nutritive medium would increase the biomass of the fungus and still maintain its acid productivity per unit mycelial weight on the previous level.

Under the influence of ultraviolet rays, aspergilli lose, most frequently, their synthetic ability for amino acids (Mishra, Nandi, 1959; Kasatkin, 1959). Our attention was directed mostly toward those amino acids which are considered "primary" for A. niger (Foster, 1950). These include glutamic acid, proline, and ornithine. They give rise to alanine, arginine, aspartic acid, and glycine. These amino acids are formed earliest in cell synthesis and provide sources for formation of the rest. Of special importance is glutamic acid, which in most organisms is the first organic product of nitrogen assimilation (Braunstein, 1957). In the further course of transamination of the α-keto acids with glutamic acid, and as the result of the carbon skeleton reconstruction of glutamic acid and other primary amino acids, the rest of the amino acids are formed. One might assume that the retardation of any enzymatic reactions which lead to primary amino acid synthesis would be most reflected in the organism's ability to build cellular material.

In this connection we studied the effect of glutamic and aspartic acids, proline, alanine, and also aqueous extracts of malt sprouts on acid formation by the A. niger T_1 variant.

METHODS

As basic nutrient medium for growing the A. niger T_1 variant we used the following defined medium: sucrose-5%; NH_4Cl-0.35%; $MgSO_4$-0.05%; KH_2PO_4-0.05%; $ZnSO_4 \cdot 7H_2O$-0.005%; $FeSO_4$-0.0025%; tap water-100 ml. To the basic medium we added amino acids in amounts of 0.01% by nitrogen, or aqueous barley malt extract. The amino acids were added individually or in combinations of two with a 1:1 ratio. The aqueous malt sprout extract was prepared as follows: 50 grams of air-dried sprouts were submerged in 500 ml water. The extraction took 20-24 hr at room temperature, followed by 3 hr at 50 C. We then added another 500 ml of water, and the mixture was autoclaved at one atmosphere. For the experiment we used the supernatant fluid in amounts of 4 ml to one liter of medium. The medium was distributed in 25–ml amounts into 50 ml Erlenmeyer flasks and inoculated with a conidial suspension of A. niger grown for four days previously on wort agar with 1.5% NaCl at 32C. The number of conidia introduced into the flask was 250 thousand/ml medium.

The fungus was cultivated at 32 C for 43 hr, until entire thin, white wrinkled films were formed. Then the culture fluid was removed from the flasks, and under the films, which were washed with sterile tap water, we introduced 20 ml of medium consisting of a 20% solution of sucrose with 0.19% NH_4Cl. The flasks were again placed in the same incubator, and the fungus was grown for another 3 days. At the end of the experiment we determined the culture fluid's acidity by titration with 0.1N

TABLE 1. Effect of Some Amino Acids on the Growth and Acid Production of the A. niger T_1 Variant

Expt. No.	Addition to basic medium	Mycelial wt.		Citric acid		
		g	% of control	g/100 ml medium	% of control	productivity, g/g mycelium
I	No addition (control)	0.324	100	13.8	100	8.5
	Aspartic acid	0.390	120	15.1	109.4	7.7
	Glutamic acid	0.450	138.8	16.0	116	7.1
	Aspartic + glutamic acid	0.477	147.2	16.2	117	6.8
II	No addition (control)	0.320	100	13.9	100	8.7
	Proline	0.383	120	13.2	94	6.9
	Proline + glutamic acid	0.417	130	16.5	118.7	7.9
III	No addition (control)	0.328	100	14.0	100	8.5
	α-Alanine	0.355	108.2	13.7	98	7.7
	α-Alanine + glutamic acid	0.426	130	15.8	113	7.4

NaOH. Simultaneously, we determined the mycelial weight. For this, the films were filtered out, thoroughly washed and dried to a constant weight at 95 C. Each experiment included 4-5 parallel flasks; the experiments were done in triplicate. The amount of acid was expressed as citric acid, since it was established that the latter comprises 92.5% of the total acid in A. niger cultures (Imshenetskii, Solntseva, Kuranova, 1960).

RESULTS

The data characterizing the effect of some amino acids on the A. niger T_1 variant's growth and acid production on sucrose-mineral medium are presented in Table 1.

From the data we clearly see that aspartic and glutamic acids, proline and alanine, when added individually to the sucrose-mineral medium, greatly improve the growth of the T_1 mutant as compared to the control medium without amino acids. The above amino acids cause an early and a more even ripening of the fungal films.

The greatest increase in biomass (by 38.8%) was observed on growing the T_1 variant with glutamic acid; then came aspartic acid, proline and α-alanine.

These compounds exerted a different influence, however, on the acid production of the A. niger T_1 variant. T_1 films grown on the basic medium with proline or α-alanine formed somewhat less citric acid in the second sugar solution than was formed by the control. Conversely, the monoaminodicarboxylic acids, glutamic and aspartic, are compounds which stimulate both the growth and the acid production of the mutant. The amount of citric acid in T_1 variant cultures grown on the second sugar solution after their growth on glutamic acid medium comprised 116%; after growth with aspartic acid, the yield was 109% as compared to the control, which was designated as 100%.

But in spite of the apparent increase of total acidity of the mutant cultures in the presence of monoaminodicarboxylic acids, the citric acid productivity of the fungus per gram of mycelium was notably lower in both cases, owing to the considerable increase in fungal biomass. One might have presumed that adding glutamic acid in combination with other amino acids that exert a smaller influence on fungal growth would cause a greater increase in fungal productivity than addition of glutamic acid alone. To test this, instead of adding one amino acid to the basic medium we added combinations of two amino acids: glutamic acid with aspartic, glutamic acid with α-alanine. The pairs were added in a 1:1 ratio; their total nitrogen content comprised 0.01% in the medium.

The most successful of the combinations thus tested was glutamic acid with proline (Table 1). In this case we observed not only a high acidity in the medium, but also the highest productivity by the fungus of citric acid per unit weight of mycelium — this productivity approached that of the control. Upon the addition of glutamic acid and aspartic acid to the medium, there was a significant increase in fungal mycelial weight (by 47%); at the same time, these films possessed the lowest citric acid productivity. As regards the total amount of citric acid in the T_1 cultures grown on media with the above-mentioned amino acids, it approximated that obtained when adding only glutamic acid. Thus, the stimulating effect can be attributed to the addition of glutamic acid alone.

The results of these experiments led to the hypothesis that an increase in productivity per unit of mycelial weight might be achieved by the addition of glutamic acid in small quantities. In this case we would expect a smaller increase in fungal biomass. Data of one experiment demonstrating the effects of glutamic acid in varying concentrations are presented in Table 2. We observe that when the concentration of glutamic acid was diluted two-fold (from 0.01% to 0.005% nitrogen), the T_1 variant culture in the second sugar solution formed the same amount of citric acid as with the original glutamic acid concentration. The increase in fungal biomass, however, was 10-15% lower in this case. Consequently, the fungus films grown on the basic medi-

TABLE 2. Influence of Varying Concentrations of Glutamic Acid on the Growth and Acid Production of the A. niger T_1 Variant in Connection with the Age of the Films on the Primary Medium.

Addition to basic medium	Film age, hr											
	43				45				47			
	mycelial wt.		citric acid		mycelial wt.		citric acid		mycelial wt.		citric acid	
	g	% of control	g/100 ml medium	productivity, g/g mycelium	g	% of control	g/100 ml medium	productivity, g/g mycelium	g	% of control	g/100 ml medium	productivity, g/g mycelium
No addition (control)	0.271	100	11.4	8.4	0.278	100	10.6	7.6	0.305	100	10.7	7.0
Glutamic acid (0.01 % N)	0.417	153	15.7	7.5	0.447	160	15.8	7.0	0.462	151	15.8	6.8
Glutamic acid (0.005% N)	0.380	140	15.4	8.1	0.382	137,3	15,3	8.0	0.413	135	15.7	7.6

um with the 0.005% glutamic acid nitrogen possessed a higher citric acid productivity per gram of mycelium – this productivity approached that of the control.

This general rule was found to hold true with T_1 films of various ages (43, 45, 47 hr)grown on basic medium with glutamic acid. The 43-hr-old films had the highest productivity.

From these experiments we can conclude that very small amounts of glutamic acid added to the basic sucrose-mineral medium are essential for stimulating growth in the ultraviolet T_1 mutant, and for maintaining its citric acid productivity per gram of mycelium at the original level.

Higher concentrations of glutamic acid, while exerting a strong influence on the fungus' growth, lower its citric acid production per unit mycelial weight.

Since the addition of pure glutamic acid cannot have widespread practical use, there arose the need to replace it with an available plant raw material possessing equal stimulating properties. With this aim, we conducted experiments using complex organic compounds as additives to the sucrose-mineral medium (we tried yeast autolysate, corn extract, malt sprout extracts). The best results were obtained with malt sprout extracts. To the basic medium we added a 5% malt sprout extract in the amount of 4 ml of extract to each liter of medium. In parallel flasks we grew the fungus on basic medium with glutamic and aspartic acids, added in amounts of 0.01% by nitrogen. Each experiment included 4-5 parallel flasks of each medium. Table 3 presents the average data of three experiments.

From Table 3 we see that malt sprout extract exerts an effect analogous to that of the amino acids – it increases the weight of the A. niger film and raises its acid production as compared to the control.

The amount of citric acid in T_1 mutant cultures grown on basic medium with malt sprout extract and with amino acids was about equal, both exceeding the control by 22-23%. Productivity of the T_1 films was higher with the malt sprout extract than with the amino acids, since the malt extract in the given concentration caused a smaller accretion of biomass than did the amino acids. We studied the amino acid composition of the malt sprout extract by paper chromatography. Separation of the amino acids was performed on the "slow Leningrad" chromatographic paper with the following solvent mixture: n-butyl alcohol, n-propyl alcohol, glacial acetic acid, water – in the ratio 5:1:1:5. The solvent was passed three times through the paper. The chromatogram was developed with a 0.5% solution of ninhydrin in acetone; for developing the proline we used a 1% isatine solution in ethyl alcohol. The results showed that malt sprout extract contains alanine, proline, aspartic acid, glutamic acid, leucine, valine, methionine, and considerably smaller amounts of glycine, tyrosine, phenylalanine, lysine, histidine, and arginine. The figure shows one of the malt sprout extract chromato-

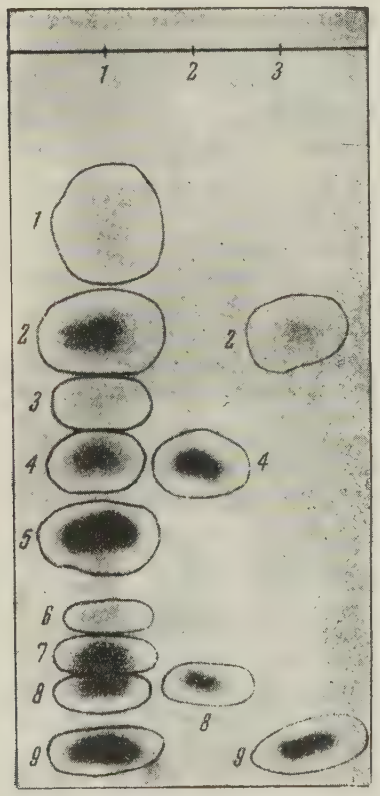
TABLE 3. Effect of Malt Sprout Extract on Growth and Acid Formation by the A. niger T_1 Variant

Addition to basic medium	Mycelial wt.		Citric acid		
	g	% of control	g/100 ml medium	% of control	productivity, g/g mycelium
No addition (control)	0.334	100	13.6	100	8.1
Malt sprout extract	0.461	138	1 .6	122.0	7.2
Glutamic + aspartic acids	0.498	149	16.7	123.5	6.7

grams. There are indications that glutamic acid and alanine comprise 44.5% of the total amino acid content of malt sprout proteins (Kretovich, 1958).

We may assume that the positive effect of malt sprout extract on the T_1 mutant growth is due to the former's high content of glutamic and other amino acids acting concomitantly. This assumption, however, does not exclude the probability that the extract's effect is also connected with other biological stimulants so abundant in plant protein.

In conclusion, I express gratitude to Prof. A. A. Imshenetskii for his advice and critical comments in connection with this work.



Malt sprout extract chromatogram. I) Contact point of extract; II, III) contact point of controls. Designations: 1) lysine, histidine, arginine; 2) aspartic acid; 3) glycine; 4) glutamic acid; 5) alanine; 6) tyrosine; 7) valine; 8) methionine; 9) leucine.

SUMMARY

1. The biomass of the ultraviolet T_1 mutant of Aspergillus niger increases by 8.20 and 38% respectively upon cultivation in a sucrose-mineral medium to which alanine, proline, aspartic or glutamic acid is added.

2. The absolute amount of citric acid in the culture of the T_1 mutant cultivated with aspartic or glutamic acid increases by 9-16% versus the basic medium. However, productivity of the film of the variant decreases versus the control with regard to formation of citric acid per unit weight of the mycelium.

3. It was shown that addition of small amounts of glutamic acid is optimal for growth stimulation of the T_1 mutant without disturbing the production of citric acid per unit mycelium weight.

4. The minimal concentration of glutamic acid exerting a favorable effect upon growth and acid formation of mutant T_1 is a concentration of 0.05%.

5. Aqueous extracts from malt sprouts exert a positive effect upon growth and acid formation of the T_1 mutant similar to that of monoaminodicarboxylic acids.

LITERATURE CITED

1. A.E. Braunshtein, 1957. Collection of Essays: "Origins of Life on Earth." AN SSSR Press.
2. A. A. Imshenetskii, L. I. Solntseva and N. F. Kuranova. 1959. Dokl. AN SSSR 124 (4): 925.
3. A. A. Imshenetskii, L. I. Solntseva and N. F. Kuranova. 1960. Microbiology, Vol. 29, No. 3.
4. I. D. Kasatikin, 1959. Microbiology, Vol. 28, No. 6.
5. V. L. Kretovich, 1958. Grain Biochemistry [in Russian] (Izd. AN SSSR).
6. D. Foster, 1950. Chemical Activity of Fungi. Foreign Literature Press [Russian translation].
7. A. K. Mishra and P. Nandi, 1959. Sci. Cult. 25, No. 1.

RIBONUCLEIC ACIDS OF THERMOPHILIC AND MESOPHILIC CULTURES OF Saccharomyces cerevisiae Strain XII

T. N. Evreinova, I. V. Marchenko, and L. G. Loginova

M. V. Lomonosov Moscow State University

Translated from Mikrobiologiya, Vol. 30, No. 3, pp. 453-456, May-June, 1961
Original article submitted July 22, 1960

Nucleic acids and other very important compounds play an active part in enzymatic and energetic cellular reactions, including protein synthesis.

In thermophilic organisms all these processes are considerably accelerated. Hence, it is quite possible that at high temperatures nucleic acids of thermophilic microorganisms undergo changes in comparison with nucleic acids of mesophilic forms. It was shown in previous papers that in optimum conditions of life and at the same stages of development the nucleic acid content was lower and the ratio of the constituent nucleotides was different in thermophilic bacteria Bacillus licheniformis (Ereinova, Bunina and Kuznetsova [1959]) and in fungi Asperigillus fumigatus (Ereinova, Maslova, Ermokhina and Sizova [1960]) in comparison with their mesophilic forms.

As the object of the present investigation we used Saccharomyces cerevisiae strain XII, a yeast employed in the alcohol industry and which grows at 28-30 C and a thermophilic variant derived from it and having a growth temperature of 39-40 C.

This yeast variant was obtained by Loginova by adaptation of the original form to high temperature and has been maintained at the above temperature for 13 years (Loginova [1960]).

As we know, when the temperature of the fermenting vats of alcohol plants is high, particularly in summer, the activity of normal yeasts is greatly reduced and in this case they can be replaced by the thermophilic variant.

For an investigation of the effect of temperature on nucleic acids mesophilic and thermophilic cultures at the same stage of development are required. This is to rule out any changes which might occur in the nucleic acids owing to the development of the microorganisms.

To obtain the yeast mass for the chemical analyses we cultured the two variants in sterile agar wort (14-15 Balling) prepared by Odintsova's method [1959].

At the end of cultivation the yeast cells were washed free of the culture fluid with tap water and then with distilled water. The wash water and culture medium were separated from the cells by centrifugation at 5000 rpm for 10 min. The sediment was ground with liquid air and was then lyophilized. The stage of development of the yeast was determined from the production of biomass. For this purpose 5 ml of agar wort in a test tube was inoculated with a known number of yeast cells (10-15 million). The test tubes were incubated for various times at 40 C for the thermophilic culture and 30 C for the mesophilic culture. At the end of incubation a fixed quantity was taken for a cell count, and the residue of lyophilized yeasts was brought to absolute dry weight in a desiccator at 105 C. The cell count was made in a Goryaev cell. Thus, knowing the weight of the cell mass and the number of cells in the weighed batch, we could calculate the weight of one cell.

From these experiments we found that the greatest biomass in the mesophilic and thermophilic cultures was produced after 36 hr of growth. It should be noted, however, that in the thermophilic culture the biomass produced after 36 hr only slightly exceeded the biomass obtained after 30 hr of growth. At the same time, the fermentative activity of the 36-hr-old thermophilic culture was higher than that of the 30-hr culture. Hence, for chemical analyses enrichment cultures of the two strains were grown for 36 hr. In this case 100 ml batches of nutrient in 500 ml Erlenmeyer flasks were inoculated with the yeasts. The state of the culture was checked from a count of live and dead cells stained with methylene blue (live cells do not stain), and from a determination of different forms of nitrogen. The cultures were in good condition, since hardly any dead cells were found. The data of the determinations of different forms of nitrogen by Barnstein's method involving distillation of ammonia by the semimicro-Kjeldahl procedure (Belozerskii and Proskuryakov [1951]) are given in Table 1.

The figures in Table 1 show that the yeasts contained mainly high-molecular nitrogenous organic substances. The low percentage of nonprotein nitrogen indicates the absence of autolytic processes (proteolysis).

TABLE 1. Content of Different Forms of Nitrogen as Percentage of Dry Weight of Yeast Mass

Culture	Total nitrogen	Protein nitrogen + nucleic acid nitrogen	Nonprotein nitrogen
Thermophiles	5.23	4.52	0.71
Mesophiles	6.84	5.99	0.85

TABLE 2. Characterization of Ribonucleic Acids of Yeasts

Mononucleotide	μM/g dry weight of yeast		Molar percentage*	
	t†	m†	t†	m†
Adenylic acid	28.73	37.45	27.9	28.6
Guanylic acid	26.28	33.49	25.5	25.6
Cytidylic acid	22.77	28.00	22.1	21.4
Uridylic acid	25.23	31 93	24.5	24.4
Total	103.01	130.87	100	100

* For calculation of the molar percentages the total sum of the mononucleotides was taken as 100%, and the fraction of each mononucleotide was expressed as a percentage of this sum.

† t) Thermophiles; m) mesophiles.

TABLE 3. Characterization of RNA According to Chargaff's Rules *

Culture	Natural $\frac{G+U}{A+C}$	Purines / Pyrimidines	Type $\frac{G+C}{A+U}$
Thermophiles	1.00	1.15	0.91
Mesophiles	1.00	1.18	0.89

* See Belozerskii and Spirin [1956]. G) Guanylic; A) adenylic; C) cytidylic; U) uridylic acid.

TABLE 4. RNA and Protein Content of One Yeast Cell

Culture	Cell weight $1 \cdot 10^{-12}$ g	Protein $1 \cdot 10^{-12}$ g	RNA $1 \cdot 10^{-12}$ g
Mesophilic	40.9	13.49	1.82
Thermophilic	31.0	7.66	1.09

NUCLEIC ACIDS

Ribonucleic acids were determined by a modified Schmidt and Thannhauser procedure, in which the quantity of nucleic acids was found and calculated from the sum of their constituent mononucleotides (Evreinova, Bunina and Kuznetsova [1959]). The latter were obtained as a mixture after hydrolysis of the whole yeast mass. The mixture was separated into the different mononucleotides by paper chromatography and paper electrophoresis. The chromatograms and electrophoresis diagrams were photographed in ultraviolet light (Evreinova, Ermolaeva and Gerasimova [1958]) and then the mononucleotides were eluted with 1M phosphate buffer at pH= 7.0 for 12 hr. The absorption of the eluates was measured by spectrophotometer and was used to calculate the concentration of mononucleotides. As an example, Fig. 1 shows photographs of the RNA mononucleotides of the thermophilic culture. Similar photographs were obtained for the mesophilic form. Details of the method and the calculation formulas were given earlier (Evreinova, Bunina and Kuznetsova [1959]). But we must repeat ourselves here, since there was a misprint in the pH of the phosphate buffer in our 1959 paper. The conditions of alkaline hydrolysis of the yeast mass are of particular importance for the quantitative determination of RNA mononucleotides. We used various concentra-

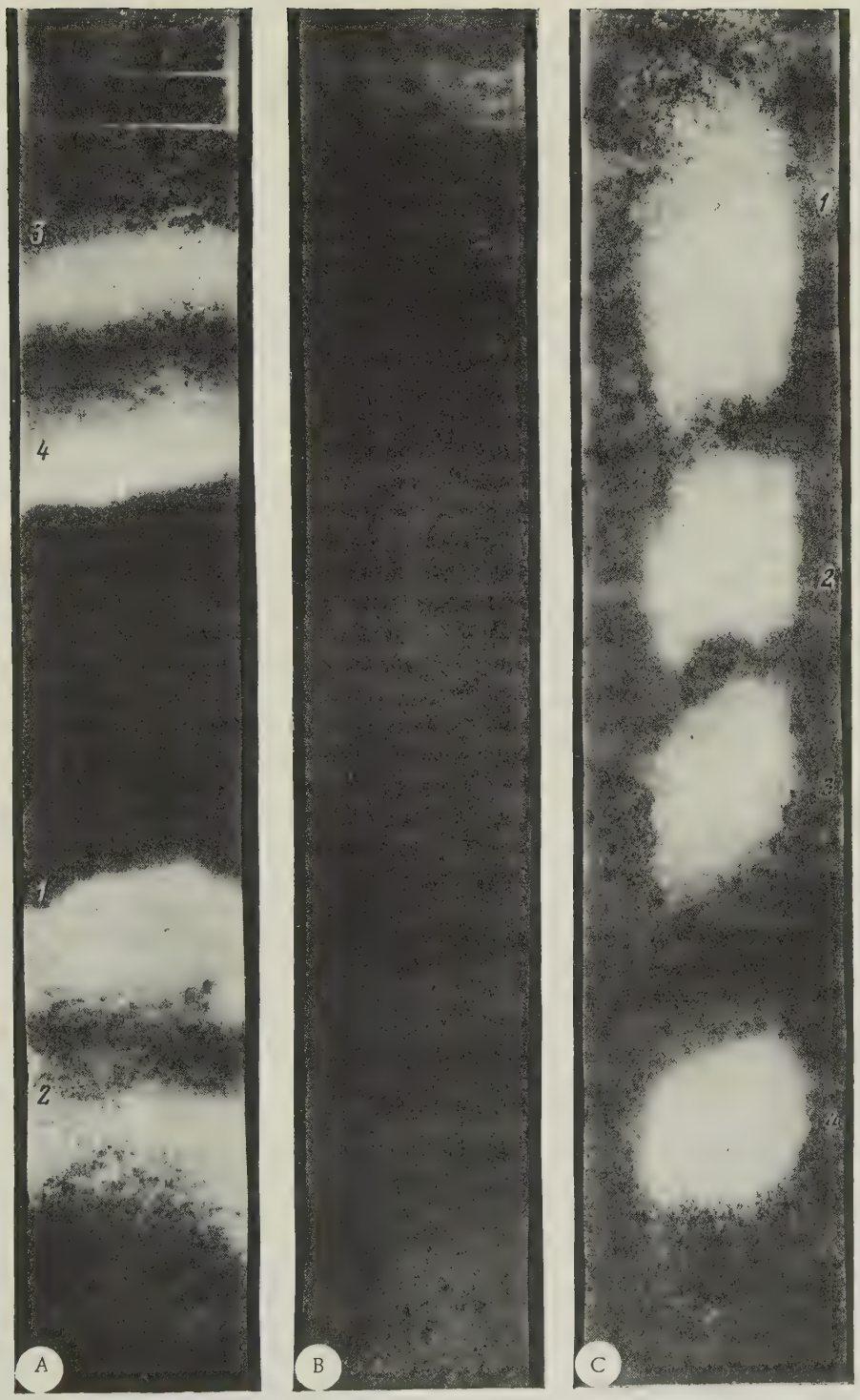


Fig. 1. Thermophilic yeast culture. A) Electrophoresis diagram. Initial weight deposited on starting line corresponded to 3.1 mg of yeasts. Separation was carried out at 860 for 4-5 hr at 0 C; B) control determination; C) chromatogram; initial weight deposited on starting line corresponded to 4.65 mg of yeast. 1) Guanylic acid; 2) uridylic acid; 3) cytidylic acid; 4) adenylic acid.

tions of alkali, from 0.3 N to 0.8 N. The best results were obtained with 0.5 N KOH for 18 hr at 37 C. In this case the mononucleotide yield for mesophilic cultures was 5% higher, and for thermophilic cultures 18% higher, than with 0.3 and 0.8 N KOH.

The mononucleotide content of the ribonucleic acids, and also the RNA composition in molar percentages and according to Chargaff's rules are given in Tables 2 and 3.

The results given in Tables 2 and 3 show that the sum of the mononucleotides in the RNA of the thermophilic culture was much smaller than in the mesophilic culture. Moreover, in the RNA of thermophiles the molar percentage of adenylic acid was less, and that of cytidylic acid more, than in the RNA of the mesophiles. The ribonucleic acids of each form of yeast belong to the A + U type, where the sum of the adenylic and uridylic acids is greater than the sum of guanylic and cytidylic. Our data agree with the determinations of Elson and Chargaff [1955], who investigated the nucleic acids of a mesophilic yeast strain. However, there is no single standard on this question, since other authors have found that yeast ribonucleic acids belong to the G + C type, where the sum of guanylic and cytidylic acids exceeds that of adenylic and uridylic (Hakim [1957]).

The percentage of nucleic acids and protein in the yeast cultures is shown in Fig. 2. In this case the protein was calculated from the difference between the total protein and the nucleic acid content. The total protein was calculated, as usual, from the protein nitrogen, which also includes the nucleic acid nitrogen. The protein and nucleic acid contents of one yeast cell are given in Table 4.

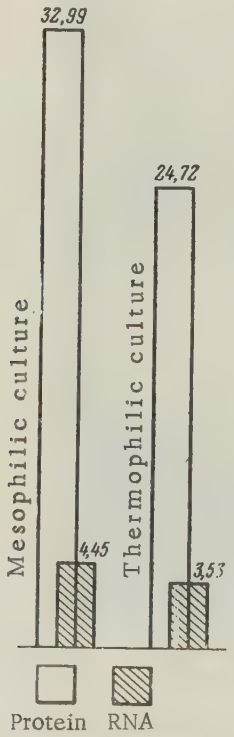


Fig. 2. Protein and nucleic acid content as percentage of absolute dry weight of yeast.

It is clear from Fig. 2 and Table 4 that when the life temperature of the yeasts is higher there is a reduction in the cell weight, the nucleic acid content and protein content. The protein content is 25% lower than that of the mesophilic yeast culture. In our previously published studies of bacteria and fungi we also observed a reduced amount of nucleic acid in thermophilic cultures, but the protein content was altered very slightly. We must note that yeasts, in contrast to other microorganisms, are extremely difficult to adapt to high temperatures. Hence, in this case we can accept it as a fact that temperature adaptation of yeasts results in a reduction of the nucleic acid content of their cells. We do not know, however, if this is due to the effect of temperature on the nucleic acids or to the effect of increased temperature on the organism as a whole. In any case there is no doubt that in the process of adaptation of yeasts to high temperature there is a radical alteration of the whole metabolism and the yeasts become capable of active metabolism at high temperatures.

SUMMARY

1. Thermophilic (growing at 39-40 C) and mesophilic (growing at 28-30 C) strains of Saccharomyces cerevisiae were investigated.

2. Nucleic acids were assayed by summation of their constituent mononucleotides.

3. The increase in temperature of life was accompanied by a reduction in nucleic acid content by 20.5%, protein content by 25%, and cell weight by 43%.

4. The ribonucleic acids of thermophilic and mesophilic cultures belonged to the A + U type, where the sum of adenylic and uridylic acids exceeds that of guanylic and cytidylic acid. In the mononucleotide composition of the RNA of the thermophilic culture the molar percentage of cytidylic acid was a little higher, and that of adenylic acid was lower, than in the mesophilic form.

5. The organic nitrogen compounds of the yeasts consisted mainly of high-molecular compounds.

LITERATURE CITED

1. A. N. Belozerskii and N. I. Proskuryakov. 1951. Practical Manual of Plant Biochemistry [in Russian] (Izd. Sov. nauka, Moscow).
2. A. N. Belozerskii and A. S. Spirin. 1956. Uspekhi Sovremennoi Biol. 41, 144.
3. T. N. Evreinova, N. N. Bunina and N. V. Kuznetsova. 1959. Biokhimiya 24, 912.
4. T. N. Evreinova, L. I. Ermolaeva and A. M. Gerasimova. 1958. Doklady Akad. Nauk SSSR 118, 334.
5. T. N. Evreinova, S. V. Maslova, T. V. Ermokhina and T. P. Sizova. 1960. Mikrobiologiya 29, 516.

6. L. G. Loginova. 1960. Physiology of Experimentally Obtained Thermophilic Yeasts [in Russian] (Izd. AN SSSR, Moscow).
7. E. N. Odintsova. 1959. Microbiological Methods of Studying Vitamins [in Russian] (Izd. AN SSSR).
8. D. Elson and E. Chargaff. 1955. Biochim. Biophys. acta 17, 367.
9. A. Hakim. 1957. J. Biol. Chem. 225, 689.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

TRANSFORMATION IN NODULE BACTERIA

R. Balassa* and M. Gabor

Institute of Genetics, Hungarian Academy of Sciences, Budapest
Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 457-463, May-June, 1961
Original article submitted October 28, 1960

The transformation of microorganisms by means of DNA is one of the lines of genetical research which enables a study of the relation between the structure of the genetic material and its function. The study of transformation involves great difficulties, since very few suitable objects, and very few of the characters required for analysis, are known.

Our previous investigations showed that certain Rhizobia can be used for the study of transformation. Beginning in 1954 Balassa used DNA preparations to endow nodule bacteria with various characters, viz., altered antigenic structure, resistance to antibiotics, nodule formation on a different host plant, acquisition of prototrophy by a cysteine-deficient mutant (Balassa [1954, 1955, 1956, 1957a, b, 1958]). The transformation of these characters was conducted on mucoid strains of Rhizobium meliloti and Rhizobium lupini. The low frequency of transformation did not provide the opportunity for a more thorough quantitative analysis.

For a study of the transformation kinetics and a fuller quantitative study of the transformation mechanism a greater frequency of transformation had to be obtained. Hence, instead of the mucoid strains, which produce an extremely large amount of polysaccharides, we used the "rough" variants in our investigations.

METHODS

Obtention of Rough Strains

Balassa succeeded (after very long unsuccessful attempts) in obtaining rough strains from the initial mucoid strain H-13 of R. lupini by ultraviolet irradiation. This initial strain was also a cysteine-deficient mutant and had also been obtained by exposure to ultraviolet. It was previously used as a recipient in the transformation of prototrophy. The two colonies obtained from the mucoid strain of R. lupini were very mutable. They became dissociated for a long time and gave rough forms. These forms differed from one another in pigmentation, degree of roughness, growth rate and times of division (Figs. 1, 2, 3). The microorganisms were cultured on a defined medium containing yeast extract and casein hydrolyzate.

The rough strains were sensitive to streptomycin. By selection from these strains we succeeded in isolating mutants resistant to high concentrations of streptomycin (500 and 1000 μg/ml). The streptomycin resistance was obtained after a single passage. The designations of the strains used in the transformation experiments are given in Table 1.

Isolation of DNA

To obtain DNA from streptomycin-resistant strains we used a modified Avery procedure (Avery [1944]). Mechanical treatment of the cells was combined with treatment with sodium deoxycholate and heating at 55 C for 20 min. The protein was removed with chloroform by Sevag's method (Sevag [1934]) and precipitated with ethyl alcohol. This procedure was repeated several times. The DNA was dissolved in citrate and sodium chloride, and the preparation was preserved by Zamenhof's method [1957]. The DNA was determined by Dische's method [1930].

Method of Transformation Experiments

One of the most important factors in transformation experiments is the control procedure.

In our investigations we first checked the sterility of the DNA preparation at different stages of isolation and purification. For the experiments we used only sterile DNA, which was repeatedly checked for sterility in the following way. A concentrated solution of the DNA was incubated in the same conditions as the recipient strain, i.e., for 24 or 48 hr at 30 C in streptomycin-free nutrient. This was followed by plating out on petri dishes containing solid medium with and without streptomycin.

When 0.1 ml of suspension (10^8 cells) of the sensitive strain was plated out on nutrient without DNA, but with 10-100 μg of streptomycin per ml, not a single colony grew in 24-72 hr.

The transformation experiments were carried out in the following manner. Two ml of nutrient medium was inoculated from a culture of the sensitive strain from an agar slant and was incubated at 30 C for 24 hr.

* Deceased.

TABLE 1. Designations of Donor and Recipient Strains of Nodule Bacteria

Microorganisms	Strains	Description of strains
R. lupini	H-13-1	Rough, streptomycin-sensitive
	H-13-2	The same
	H-13-3	The same
	$H\text{-}13\text{-}1^{1000}$	Rough, resistant to 1000 μg of streptomycin per ml
	$H\text{-}13\text{-}2^{1000}$	The same
	$H\text{-}13\text{-}3^{500}$	Rough, resistant to 500 μg of streptomycin per ml
	$H\text{-}13\text{-}T3^{1000}$	Rough, acquired resistance to 1000 μg of streptomycin per ml in course of transformation
	$H\text{-}13^{1000}$	Streptomycin-resistant, initial mucoid variant
R. meliloti	$M\text{-}32^{10000}$	Streptomycin-resistant mucoid variant

Note: Index indicates streptomycin concentration tolerated by strain.

A description of the donor and recipient strains is given in Table 1. In this case the number of cells in the suspension was 10^7/ 0.1 ml. The culture was diluted a hundredfold in new nutrient. Thus, we obtained a young culture, which after a short (1-2 hr) lag period continued to divide. Sometimes a synchronized culture was required for the experiments. The diluted culture was then kept at 4 C in a refrigerator.

The start of the experiment was taken as the moment when the culture was diluted a hundredfold. In the case of a synchronous culture the start of the experiment was taken as the moment when the culture was installed in the incubator. The experiment was stopped after 24 or 48 hr by plating out the culture on a selective medium, which usually had a streptomycin content of 100 μg /ml. Sometimes the action of the DNA was stopped by deoxyribonuclease in concentration 25 μg/ml. In this case the sterility of the deoxyribonuclease was checked separately.

RESULTS

We first compared the ability of different rough strains of R. lupini to undergo transformation. The frequency of transformation in strain H-13-1 under the action of DNA of strain $H\text{-}13\text{-}1^{1000}$ was low (1×10^{-7}). With strain H-13-2 under the action of DNA of strain $H\text{-}13\text{-}2^{1000}$ we obtained no transformations at all, whereas in strain H-13-3 under the action of the DNA of $H\text{-}13\text{-}3^{500}$ we observed a high frequency of transformation (0.1-0.5%). Hence, only strain H-13-3 was used in subsequent transformation experiments with DNA. The DNA preparations were obtained from various resistant strains belonging to the species R. lupini (in experiments on intraspecific transformation) and R. meliloti (in experiments on interspecific transformation) (Table 2).

The DNA of $H\text{-}13\text{-}3^{500}$ is homologous for strain H-13-3. The DNA of $H\text{-}13\text{-}T3^{1000}$ was obtained from a culture isolated from a colony which had become resistant to streptomycin after transformation (Table 1). As we see, the DNA of the latter culture gave a higher frequency of transformation than the initial DNA of the resistant strain. This agrees well with Schaeffer's results [1956].

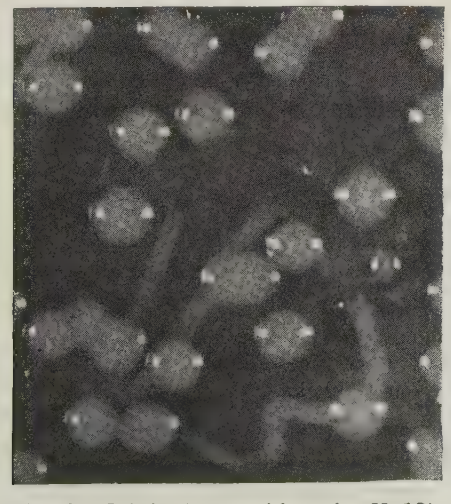


Fig. 1. Original mucoid strain (H-13) of Rhizobium lupini.

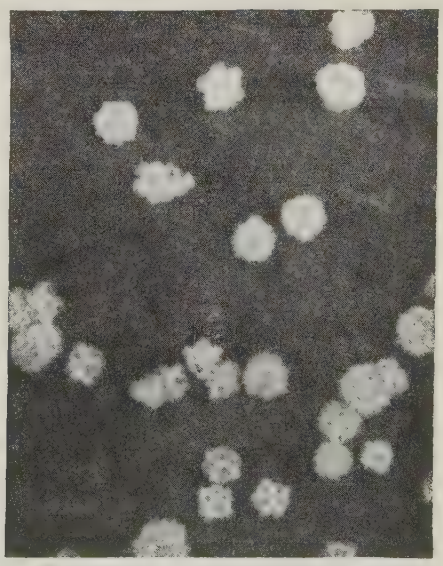


Fig. 2. Rough strain (H-13-2) of Rhizobium lupini obtained by exposure to ultraviolet.

TABLE 2. Intra- and Interspecific Transformation (DNA given at start of expt. Cultures plated out after 24 hr. DNA recipient – strain H-13-3. Initial number of cells $5.5 \cdot 16^{-6}$/ml, final number $-2.4 \cdot 10^{-8}$/ml)

DNA donor strains	Transformants/0.1 ml
H-13-3^{500}	690
H-12-$T3^{1000}$	2800
H-13-2^{1000}	90
H-13-1^{1000}	10
H-13^{1000}	30
M-32^{10000}	2

TABLE 3. Relation Between Frequency of Transformation and DNA Concentration (DNA added at start of expt. Cultures plated out on selective medium after 24 hr. Recipient strain H-13-3, DNA donor strain H-13-3^{500}. Initial number of cells $-3 \cdot 10^{5}$/ml, final number $-1.7 \cdot 10^{7}$/ml)

DNA concentration, μg/ml	Transformants/0.1 ml
15	62
1.5	65
$1.5 \cdot 10^{-1}$	190
$1.5 \cdot 10^{-2}$	47
$1.5 \cdot 10^{-3}$	12
$1.5 \cdot 10^{-4}$	2

Intraspecific transformation by heterogeneous DNA obtained from strains H-13-1^{1000} and H-13-2^{1000} was much less frequent. It should be noted that strain H-13-1^{1000} was not only streptomycin-resistant but was also streptomycin-dependent. The results on the transmission of this property, a new character in transformation studies, were reported at the Third Congress of Hungarian Microbiologists (Balassa and Gabor). Transformation with M-32^{10000} DNA is of particular interest, since this is interspecific transformation (Table 1). With such transformation we obtain a fundamentally new method of identifying bacteria, since transformation occurs only between closely related species.

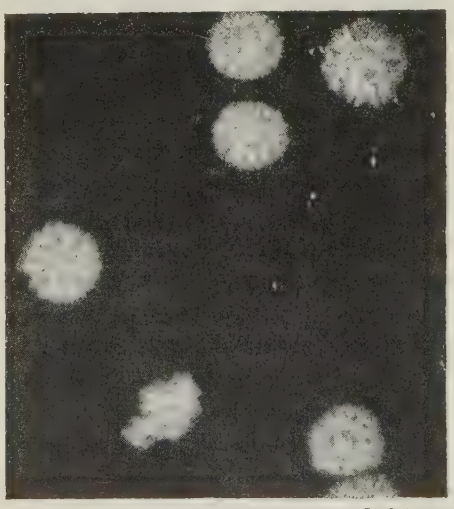


Fig. 3. Rough strain (H-13-3) of Rhizobium lupini obtained by exposure to ultraviolet.

We next determined the relationship between transformation and DNA concentration (Table 3).

The optimum concentration was 0.15μg DNA per ml.

To determine the time required for DNA adsorption we added it to a synchronized culture after 12 hr, and arrested its action with deoxyribonuclease after 0, 10, 30 and 60 min, respectively (Table 4).

The optimal adsorption time was 30 min. We cannot yet explain the smaller number of transformants after exposure to DNA for 60 min.

To decide if there was a period of competence (i.e., a special physiological state when cells are subject to transformation) in the bacterial life cycle, we had to synchronize the cell division, as we mentioned already in the methods section. The start of the experiment was taken as the moment when the population was installed in the incubator. The DNA was added after 12, 16 and 20 hr. The action of the DNA in each case was stopped by deoxyribonuclease after 30 min. The cells were plated out at the end of the experiment (i.e., after 24 hr) on nutrient medium with a streptomycin content of 80 μg/ml (Table 5).

TABLE 4. Time Required for DNA Adsorption (DNA donor – strain H-13-3^{500}, recipient – strain H-13-3. Time of addition of DNA – 12 hr, incubation period – 24 hr. Initial number of cells – $2 \cdot 10^5$/ml. Number of cells after 12 hr – $5.2 \cdot 10^6$/ml, final number – $2.3 \cdot 10^7$/ml)

Adsorption time, min	Transformants/0.1 ml
0	0
10	0.5
30	240
60	39

TABLE 5. Relation between Frequency of Transformation and Age of Cells (DNA donor–strain H-13-3^{500}, recipient–H-13-3. Incubation period 24 hr. Number of cells: initial – $2 \cdot 10^5$/ml, after 12 hr $5.2 \cdot 10^6$/ml, after 16 hr – $3.6 \cdot 10^6$/ml, after 24 hr – $2.3 \cdot 10^7$/ml)

Culture age when DNA was added, hr	Transformants/0.1 ml
12	240
16	0.5
20	0

TABLE 6. Relation Between Number of Transformations and Time of Addition of DNA (DNA donor – strain H-13-3^{500}, recipient – H-13-3. Incubation period – 8 hr. Initial number of cells – $2.4 \cdot 10^5$/ml, after 8 hr – $3.2 \cdot 10^6$/ml)

Culture age when DNA was added, hr	Transformants/0.1 ml
0	103
1	750
2	1600
4	125
6	—
8	—

As the given data show, there was no transformation during the second half of the experiment. Apparently the physiological state – the competence of the cells – is an important factor in transformation. We were interested in the role played by the retardation in the phenotypic manifestation of streptomycin resistance. In view of our observation that bacteria are in a competent state for transformation during the first half of the life cycle, the experiment was conducted during the first 8 hr. A 24-hr old culture was diluted fiftyfold, and not a hundredfold, with fresh nutrient. In every case the cultures were plated out on agar 8 hr after the start of the experiment.

As Table 6 shows, we obtained the highest number of transformants when DNA was added 2 hr after the start of the experiment and acted for 6 hr. The smallest number of transformants, which was obtained when DNA acted for 4 hr, and the complete absence of transformation when DNA acted for 2 hr or when DNA was added at the time of plating out, can be attributed not only to the physiological state of the cells but also to the fact that there is a delay in the consolidation and phenotypic manifestation of the newly acquired property.

TABLE 7. Degree of Transmitted Streptomycin Resistance in Relation to DNA Donor (Recipient – strain H-13-3, DNA added at start of expt; cultures plated out on selective medium after 24 hr. Initial number of cells – $5.5 \cdot 10^6$/ml, final number – $1.6 \cdot 10^8$/ml)

DNA donor	Transformants/0.1 ml at streptomycin concentration of μg/ml		
	10	100	1000
H-13-T3^{1000}	460	300	180
H-13-2^{1000}	180	97	8

In our investigation of the level of streptomycin resistance transmitted we obtained the data in Table 7.

Strain H-13-T3^{1000} DNA, obtained from a transformed colony which had become resistant to 1000 μg strepto-

TABLE 8. Transmission of Streptomycin Resistance in R. lupini (Donor – strain H-13-3^{500}, recipient – strain H-13-3. Cultures plated out on selective medium after 24 hr. Initial number of cells – $2 \cdot 10^5$/ml, after 12 hr – $5.2 \cdot 10^6$/ml, final number – $2.3 \cdot 10^7$/ml)

Culture age when DNA was added, hr	DNA adsorption time, min	Transformants/0.1 ml at streptomycin concentration of μg/ml					
		10	50	80	100	500	1000
12	10	4,5	2	2	2	0.5	1.5
	30	380	150	190	180	110	95
	60	81	62	48	35	28	18
20	10	7	—	—	—	—	—
	30	1	—	—	—	—	—
	60	0.5	—	—	—	—	—

mycin per ml, transmitted to sensitive cells a resistance of the same order, as revealed on different concentrations of streptomycin, whereas the DNA of the other strain gave a resistance reduced by two orders. The level of resistance transmitted by the DNA of strain H-13-3^{500} (Table 8) was higher than that of the donor strain.

As we see, the homologous DNA of the initial resistant mutant H-13-3^{500} transmits a resistance of the same order, although at higher streptomycin concentrations fewer transformants appear.

SUMMARY

1. Interspecific and intraspecific transformation with frequency $30/10^7$ and $2/10^7$ was obtained by the use of DNA from streptomycin-resistant mutants.

2. A higher frequency of transformation ($690/2.4 \cdot 10^7$) was obtained with intrastrain homologous transformation.

3. DNA obtained from a strain which had become resistant to streptomycin by transformation gave an even higher frequency of transformation – $2800/10^5$.

4. DNA obtained from strain H-13-3^{500} in concentration 0.15 μg/ml gave the greatest number of transformants.

5. The optimal time for DNA adsorption is 30 min.

6. Bacterial transformation depends on the age and competence of the recipient cells.

Phenotypically the transformation change is manifested gradually.

7. By induction with homologous DNA obtained from a streptomycin-resistant strain, the same degree of resistance as that of the donor strain is transmitted. On exposure to DNA from another strain a lower level of resistance is transmitted.

LITERATURE CITED

1. O. T. Avery, C. M. MacLeod and M. McCarty. 1944. J. Exp. Med. 79, 137.
2. R. Balassa. 1954. Acta Microbiol., A. Sci. Hung., II. Fasc. 1-2.
3. R. Balassa. 1955. Naturwiss. 42, 14, 422.
4. R. Balassa. 1956. Naturwiss 43, 6, 133.
5. R. Balassa. 1957a. Acta Microbiol., A. Sci. Hung., IV, Fasc. 1, 77.
6. R. Balassa. 1957b. Acta Microbiol., A. Sci. Hung., IV, Fasc. 1, 85.
7. R. Balassa. 1958. Abstr. Intern. Congr. Microbiol., VII Congr., p. 49.
8. Z. Dische 1930. Microchemie 8, 4.
9. P. Schaeffer. 1956. Ann. Inst. Pasteur 91, 192.
10. M. G. Sevag. 1934. Biochim. Z. 273, 419.
11. St. Zamenhof. 1957. Chemical Basis of Heredity, Baltimore, p. 351.

INTERSPECIES TRANSFORMATION IN MICROORGANISMS

A. A. Imshenetskii and S. É. Mansurova

Institute of Microbiology, Academy of Sciences, USSR
Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 464-465, May-June, 1961
Original article submitted October 19, 1960

In an earlier publication Imshenetskii and Perova (1960) reported on the transfer of resistance to streptomycin from Staphylococcus aureus strain 209, resistant to 40,000 units of streptomycin per ml of medium, to the original non-resistant strain by means of DNA-containing extracts. The resistant strain was obtained through serial passaging in a medium containing increasing concentrations of streptomycin. In this case the transformation occurred among closely related organisms.

There is at present an increasing amount of data on the fact that in certain cases interspecific transformation is possible. It was at first shown for the genus Haemophilus (Alexander and Leidy, 1955; Schaeffer and Ritz, 1955; Schaeffer, 1956, 1957; Schaeffer, 1960). The frequency of transformation of streptomycin resistance to Haemophilus influenzae by means of DNA from the resistant strain of H. parainfluenzae was 10^4 times less than when the DNA was obtained from a resistant strain of H. influenzae (Schaeffer, 1956). Timakov and Semcheva (1956), Semcheva (1956), Gadzhieva (1956), Kudlai (1956), Kudlai et al. (1956) studied the experimental variability of different species of bacteria of the coli group, induced by cultivation on a medium containing heat killed cells or nucleoproteins. The possibility of interspecific transformation was shown for nodule-forming bacteria (Balassa, 1957; Balassa and Gabor, 1961; Krasil'nikov, 1945). Finally, Bracco et al. (1957), and then Pakula et al. (1958), reported on the transformation between different representatives of genera of the family Coccaceae. The last named authors transmitted resistance to streptomycin to non-resistant cultures by means of DNA from the streptomycin-resistant cultures. The resistance was transmitted from pneumococci to all the strains of streptococci tested. When DNA from staphylococci was used, only 2 of 83 strains of streptococci tested became transformed. Resistance could not be transmitted to pneumococci by means of DNA from staphylococci and from enterococci.

We have attempted to transmit streptomycin resistance to non-resistant cultures of Micrococcus sulfureus, M. carneus, M. rubifaciens, M. citreus and the systematically remote species Escherichia coli by means of an extract obtained from a strain of S. aureus strain 209, resistant to 40,000 units of streptomycin per ml of medium. The non-resistant cultures were grown for 24 hr at 37C on meat-peptone agar, then transferred to meat-peptone broth. Approximately 8 hr cultures on the last named medium were used for the transformation experiments. Equal volumes of the diluted extract and the cultures were placed in an incubator at 37C for 24 hr. The number of resistant cells was determined by plating on meat-peptone agar in petri dishes containing 40,000 units of streptomycin per ml. This method was described in detail previously (Imshenetskii and Perova, 1960). The petri dishes were examined after 24, 48 and 72 hr. Each experiment was repeated 3 times. The original non-resistant culture of S. aureus was used as control. The results of these experiments are shown in the table.

The table shows that in all cases except the last (in which the resistance was transmitted to cells of the same species) the transformation to resistance did not take place. The number of resistant cells in the non-resistant culture of S. aureus increased considerably, and was equal to 3×10^{-4}.

We have pointed earlier to the successful interspecific transformations. In all cases, with the exception of the family Coccaceae, the species studied were very closely related. According to literature, transformations, as a rule, are possible only between closely related microorganisms, often only between certain strains of a given species, while numerous species cannot be transformed at all. The results of our work also point to the fact that, with the methods used, interspecific transformation among the species investigated is impossible.

SUMMARY

It was impossible to transmit resistance to streptomycin to cultures of Micrococcus citreus, M. rubifaciens, M. carneus, M. sulfureus and Escherichia coli by means of extracts obtained from cultures of Staphylocceus aureus strain 209 resistant to 40,000 units of this anti-

Results of Experiments on Interspecific Transformation

Microorganisms	Resistant mutants			Streptomycin-resistant cells after DNA treatment		
	cells growing on streptomycin-free MPA	cells growing on MPA +streptomycin 40,000 units/ml	mutants	cells growing on streptomycin-free MPA	cells growing on MPA +streptomycin 40,000 units/ml	Streptomycin-resistant variant formation frequency after DNA treatment
E. coli	$2 \cdot 10^{11}$	0	0	$1.2 \cdot 10^{10}$	0	0
M. citreus	$6 \cdot 10^{9}$	0	0	$0.3 \cdot 10^{9}$	0	0
M. sulfureus	$1 \cdot 10^{10}$	0	0	$1 \cdot 10^{9}$	0	0
M. carneus	$9 \cdot 10^{9}$	0	0	$0.8 \cdot 10^{9}$	0	0
M. rubifaciens	$2.3 \cdot 10^{9}$	0	0	$1.8 \cdot 10^{9}$	0	0
Staph. aureus 209	$1 \cdot 10^{9}$	1	$1 \cdot 10^{-9}$	$1.9 \cdot 10^{6}$	72	$3.1 \cdot 10^{-4}$

biotic per ml of medium. Thus, interspecific transformation could not be attained under our experimental conditions.

LITERATURE CITED

1. R. Balassa and M. Gabor, 1961. Microbiology 30 (3): 457.
2. G. R. Gadzhieva, 1956. The Variability of Microorganisms, Medgiz, Moscow, p. 90.
3. A. A. Imshenitskii and K. Z. Perova, 1960. Microbiology 29: 505.
4. N. A. Krasil'nikov, 1945. Microbiology 14: 230.
5. D. G. Kudlai, 1956. The Variability of Microorganisms, Medgiz, Moscow, pp. 53, 64, 75.
6. D. G. Kudlai, A. G. Skavronskaya, G. S. Soikina and N. A. Tarkhanova, 1956. Ibid., p. 83.
7. N. S. Semcheva, 1956. Ibid., pp. 38, 45.
8. V. D. Timakov and N. S. Semcheva, Ibid., p. 23.
9. P. Schaeffer, 1960. Biological Replication of Macromolecules. Publ. Foreign Lit., p. 96.
10. H. E. Alexander and G. Leidy, 1955. Amer. J. of Child Diseases 90, 560.
11. R. Balass, 1957. Acta microbiol. hung. 4, 77.
12. R. M. Bracco, M. D. Krauss, A. S. Roe and C. M. D. MacLeod, 1957. J. Exp. Med. 106, 247.
13. R. Pakula, E. Hulanicka and W. Walczak, 1958. Bull. Acad. polon. sci., ser. sci. bioi. 6, 235.
14. P. Schaeffer, 1956. Ann. Inst. Pasteur 91, 192.
15. P. Schaeffer, 1957. C. R. Acad. sci. Paris 240, 375.
16. P. Schaeffer and E. Ritz, 1955. C. R. Acad. sci. Paris 240, 1491.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-tc-cover English translations appears at the back of this issue.

EFFECT OF DISINFECTANTS ON BACTERIAL CELL MORPHOLOGY AND BIOLOGY

A. M. Malchenkov

Leningrad Military Medical Academy
Translated from Mikrobiologiya, Vol. 30, No. 3, pp. 466-472, May-June, 1961
Original article submitted January 12, 1960

We (Malchenkov and D'yakov, 1957) suggested that the dark-field method might be applicable to microbiology. We made a comparative study of the value of phase-contrast versus dark-field contrast microscopy in which the latter shows a number of advantages (D'yakov, Ivanova, and Malchenkov, 1959) which permit elucidation of even minute differences in optical density between parts of the protoplasm and create the possibility of visualizing the finer cell structures, delineating the cell membrane more clearly and obtaining photographs with better contrast.* Therefore, for our study we used the dark-field contrast method but for comparison included a number of preparations studied by bright-field phase-contrast.

The present work aimed to study the structural changes that arise in microbial cells exposed to disinfectants. All observations were performed in a Peshkov chamber.

We studied the effect of furacilin [5-nitro-2-furalde hyde semicarbazone], phenol, formalin, corrosive sublimate, hydrogen peroxide, rivanol, and hexachlorophene. In experiments on the rapid determination of bactericidal and bacteriostatic concentrations we coupled the dark-field method with a study of the microbes in the Peshkov chamber. All experiments were controlled by preliminary study of the growth of intestinal bacilli on agar in a Peshkov chamber. In control preparations the cells did not change during the lag-phase of growth, which lasted one to two hours; only at the end of the lag-phase did their optical density increase and swelling begin. Toward the start of the logarithmic phase of growth the cells slowly increased in size and began to divide so that colonies were seen within two to three hours after the Peshkov plates were seeded. Single cells resembled short rods with rounded ends, had clearly delineated membranes and were all of the same optical density (Figs. 1 and 2).

RESULTS

The effect of furacilin was studied in the Peshkov chamber by phase contrast (Figs. 3 and 4). Parallel study of various concentrations of furacilin confirmed the hypothesis that the concentration of the chemical agent substantially affects the development of and the extent of changes in the microbial cells. At high concentrations of furacilin (1:50,000) we observed sharp inhibition of cell growth.

Observation in the Peshkov chamber every 15-20 min revealed no change in the cell. The lag-phase of growth lasted 10-12 hr and only after this interval did individual cells elongate and become wider; their optical density also increased. Later, differentiation of the cellular substance became noticeable: the cytoplasm began to clear and polar granules appeared. Particles were seen in the cytoplasm, some coarse, optically dense, formless structures and some small, powder-like, dark particles. The cell membrane gradually lost its clear outline and became looser; it lost elasticity and as a result bulges, swellings and even tears appeared, through which the cytoplasm, granules and particles flowed out.

In lower concentrations (1:75,000) the lag-phase was shorter (8 to 10 hr), after which signs of growth appeared. The observation of growing cells showed that at the beginning of the logarithmic growth phase the process of cell division is paralyzed or temporarily halted in the majority of cells but growth processes are preserved. In such cases microcolonies form, consisting of masses of interlaced filaments or tendrils of various shapes. These filamentous forms subsequently undergo all stages of degeneration and disintegrate.

We often observed the formation of giant filaments containing cytoplasmic vacuoles and polar granules that differentiated into particles or amorphous granularity and appeared as optically dense, dark-colored particles by phase-contrast. On prolonged, repeated observation it was noted that these degenerative processes intensified, the cells disintegrated and in their place only membrane ghosts and debris remained.

Further diminution of the furacilin concentration (1:100,000) revealed a shorter lag-phase of 5 to 6 hr be-

* A favorable appraisal of this method has been given by Peshkov (1955), Rukavtsev (1958), Frolich (1955), Wilsca (1953, 1954), and others.

fore cell growth began. At this concentration, elongation of the cells was inhibited less and the cells divided, forming microcolonies. After 24 hr, or somewhat earlier or later, the majority of these colonies ceased to grow and quickly began to degenerate and disnitegrate. Such colonies usually turned into debris by 48-72 hr. Thus, the concentration of disinfectant determines the duration of the lag-phase for the culture.

Different degrees of structural-morphological alterations in the microbial cells appeared in different concentrations of disinfectant. Higher concentrations evoked more acute changes, that is, the granularity was more poorly differentiated and was present either as large blocks or as amorphous masses of different sizes; the cell outlines were less clear; the membrance was looser and less elastic, with a broken surface and often with bulges or tears. Most cells disintegrated. Observations by phase-contrast or dark-field methods permit one to be certain of the extremely wide range of cell sensitivity to the disinfectant within one and the same cell population. Therefore, it is important to study not the single cell but groups of them in order to avoid error.

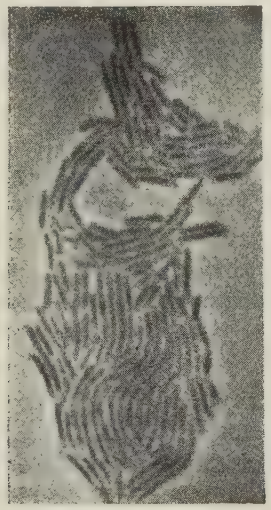


Fig. 1. Control. Three-hour colony on agar. Phase-contrast. Magnification in Figs. 1-8 1400 ×.

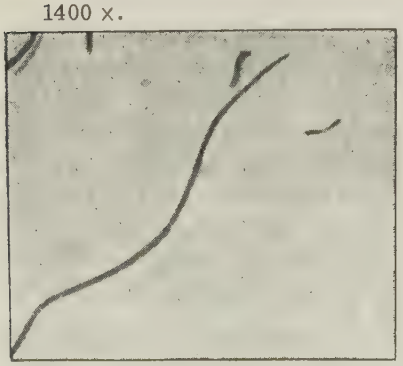


Fig. 3. Eight-hour cell-filament on agar with furacilin 1:100,000. Phase-contrast.

The specific action of furacilin on cells with high sensitivity was manifested as follows: cells usually remained unchanged for a long time as if in a state of "anabiosis" or "paralysis." Their structure was that of a homogeneous, optically dense, clearly demarcated membrane; under phase-contrast the cells resembled short, greyish rods. Such a state of anabiosis lasted two to three days. Then a decrease in contrast appeared; the cytoplasm cleared and degeneration set in. The degenerative process advanced rapidly and by the sixth or seventh day the cells disintegrated, forming masses of membrane ghosts of such low optical density that they were scarcely perceptible in photographs. In the least sensitive cells a short period of slow growth followed the lag-phase and the cells might enlarge from two to three up to eight or ten times, but the process of cell division appeared to be arrested and the cells did not divide. Soon the growth processes ceased and degenerative changes began to appear, probably immediately upon the cessation of fermentative processes. Because of this, the dispersion of the colloidal structure of the cytoplasm was disturbed and disintegration of the chromatin substance began.

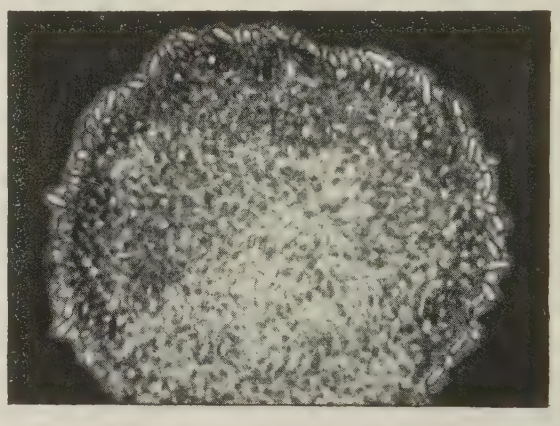


Fig. 2. Control. Four-hour colony on agar. Dark field contrast.

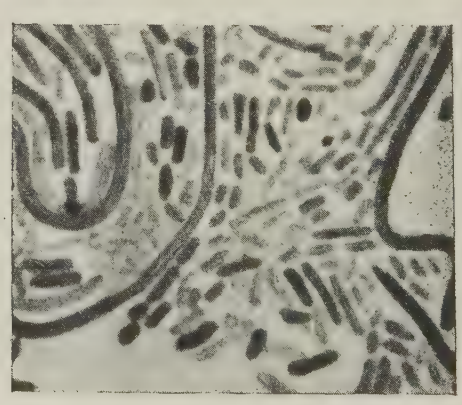


Fig. 4. Three-hour colony on agar with furacilin 1:75,000. Phase-contrast. Division of filamentous forms and dissociation of cells into membrane ghosts and debris is seen.

We consider that the finely dispersed nature of the chromatin substance underlies the optical homogeneity of the cytoplasm. The action of harmful factors that alter the colloidal state of the cytoplasm is to cause coagulation of the chromatin substance with the formation of granules, particles, agglutinated flakes, powder-like granularity and other shapeless formations. High concentrations of furacilin evidently immediately disturbed a number of vital processes including cell division and growth and evoked marked disturbances in the colloidal state of the cytoplasm, as a result of which the degenerative processes described above set in. Study of the effects of furacilin on the microbial cell in the Peshkov chamber by the phase-contrast method has significantly expanded the concept of structural-morphological changes in the cells.

The effect of phenol on the bacterial cell was studied by the same methods and confirms how important the concentration of the agent is.

On agar containing phenol 1:750 (the bacteriostatic concentration) the lag-phase of growth lasted 10-12 hr, after which an insignificant swelling and increase in optical density occurred, followed by a slow increase of cell length two or three times, but cell division did not begin and growth was halted. Then structural changes in the cells, that is, differentiation of the chromatin substance into granules, cytoplasmic granularity, loosening of the cell membrane, and so forth, gradually appeared. All of these pathological changes slowly were intensified and the cells were brought to disintegration, so that by the eighth to twelfth day only the cell membrane and debris remained.

With sub-bacteriostatic concentrations (1:800) the lag-phase of growth was shorter (6 to 8 hr); after this, swelling, darkening of the contents and elongation of the cells began. Most cells did not divide and grew instead, forming heaps of filaments. Thus, growth was arrested, polar granules slowly appeared, the optical density decreased and differentiated granules appeared in the cytoplasm. Some of these filaments divided on the third to fourth day. In others, division was delayed until the eighth to tenth day. Subsequently, all colonies underwent disintegration with the formation of debris.

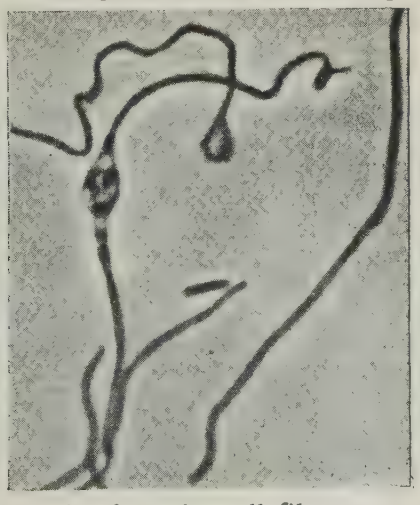


Fig. 5. Three-day cell-filament on agar containing phenol 1:900. Cytoplasmic granularity, loosening of the cell membrane, the formation of ampullae and dilatations are seen. Phase-contrast.

Further decrease of the phenol concentration to 1:900 indicated the weakened effect of this agent. The lag-phase did not exceed four hours and following it the cells displayed all signs of growth. A greater number of cells retained the ability to divide, although in some cells division was arrested temporarily; normal colonies were then formed.

As with the furacilin studies, we observed a significant variation in the range of sensitivity of cells to phenol manifested only in sub-bacteriostatic concentrations, since in bacteriostatic concentrations the cells could not react, being in an "anabiotic" or "inhitited" state. Further development of filamentous cells was almost indistinguishable from the above-described process of degeneration, but the cytoplasmic coagulation was less gross.

The specific action of phenol was shown chiefly by the significant disturbances in the cell membrane, namely, its loosening, the loss of definite outline and elasticity, the fragility and formation of ampoule-like dilatations, outpouchings, tears and disintegration (Fig. 5). Thus, the action of phenol brought about a lengthening of the lag-phase, disturbance and arrest of cell division and then even the cessation of growth. These deranging alterations probably give rise to a disturbance in the dispersion of the cytoplasmic colloids and of the cell membrane which were manifested in the destruction of the cell (Aleksandrov, 1948; Milyavskaya, 1951; Nasonov and Aleksandrov, 1940).

We studied the effect of formalin on the bacterial cell by the dark-field contrast method, which has been used also throughout this entire investigation. The effect of formalin was manifested in 1) lengthening of the lag-phase of bacterial growth (in formalin concentration of 1:12,000 the lag-phase was 8-12 hr; in concentration of 1:15,000 it was 4-6 hr); 2) the derangement of cell division in sensitive cells and the inhibition of cell division

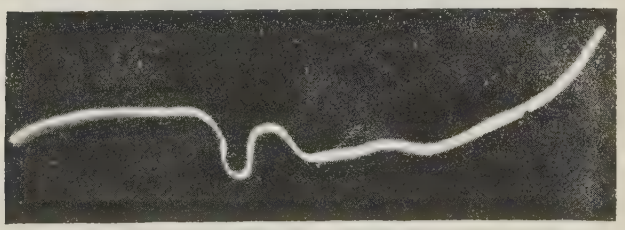


Fig. 6. Filamentous cell, aged 24 hr, on agar containing formalin 1:15,000. Dark-field contrast. The cell membrane is loosened, pitted and not clearly delineated. The cytoplasmic structure is optically non-homogeneous.

in less sensitive ones (Fig. 6); 3) the arrest and complete disruption of growth function with the appearance of degenerative processes. The specific action of formalin was manifested in a much greater effect on the cytoplasm than on the cell membrane. A varying degree of cell sensitivity to the disinfectant was also demonstrated in this experiment. Thus, highly sensitive cells after two to three days no longer showed signs of growth but degenerated. Less sensitive cells underwent two or three division cycles within 12-18 hr, after which they rapidly ceased to grow and began to degenerate. Other cells formed filaments and after some delay began to divide and form colonies. We often noticed that in apparently completely normal colonies one cell would suddenly begin to form a filament. Such a filamentous cell subsequently would go through all stages of degeneration and disintegrate.

The effect of sublimate. In bactericidal concentration (1:100,000) we saw neither growth nor cell division, but within eight to ten hours structural changes began to appear. At first the cytoplasm condensed at the poles, then polar granules appeared. Later on, the entire cytoplasm took on a fine granularity, larger particles appeared and shapeless structures. The cell membrane became less clearly outlined and the membrane itself loosened and swelled. Over three days the cells exhibited no signs of growth, but degenerative processes intensified and the cells were gradually destroyed. By the seventh to eighth day only membrane ghosts and debris remained in their stead.

With a bacteriostatic concentration (1:150,000) the lag-phase of growth was prolonged to 8 to 10 hr, after which time some cells (the less sensitive) became swollen, increased in optical density and grew longer and wider, but after the completion of three or four division cycles they stopped growing. The remaining cells were unchanged. After 24 hr the cells which had divided became clearer, granules formed at the poles and degenerative changes set in. By the sixth to eighth day these resulted in the complete disintegration of the cells. A sub-bacteriostatic concentration (1:200,000) evoked less marked changes: the lag-phase lasted less than 5 to 6 hr and a more significant number of cells had divided. In another sub-bacteriostatic concentration (1:250,000) the lag-phase of growth did not exceed 4 hr. The number of dividing cells was still greater and the microcolonies formed were larger (up to 20-40-60 cells). Sometimes the colonies filled the entire microscopic field. In still weaker concentration, up to 1:300,000 and 1:350,000, the lag-phase approached normal and did not exceed 3 to 4 hr. The number of dividing cells was still greater; in relation to this, the division time was also shorter.

The effect of sublimate on the cell further decreased at concentrations of 1:400,000 and 1:450,000. The lag-phase of growth was 2 to 3 hr. The sensitivity of the cells in this experiment also varied within wide limits; we saw colonies of different ages and sizes in a single microscopic field. The speed of cell division also varied: in less sensitive cells the division cycle ran 25-30 min, in sensitive ones 40-60 min, and in highly sensitive cells 60-80 min.

The small colonies which developed (from sensitive cells) consisted of cells which were morphologically altered: they were swollen, twisted and resembled vibrios, pyriform, ameboid and almost shapeless forms reminiscent of L-forms. Their internal structure gave the characteristic pathological picture described above [the presence of polar granules, coarse or fine granularity or shapeless masses of chromatin, loosening of the cell membrane and its deformation and rupture, and the disintegration of the cells (Fig. 7)].

Consequently, the specific action of sublimate was marked on both the cell membrane and the cytoplasm.

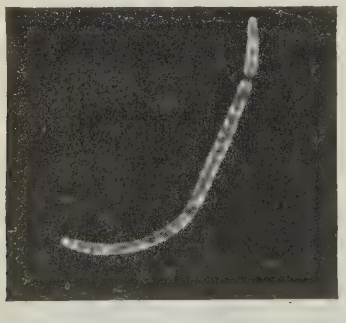


Fig. 7. Two-day cell in agar containing sublimate 1:350,000. Dark-field contrast. The outline of the cell membrane appears unclear, with serrations and pitting. Polar granules are clearly shown, the chromatin substance is granular, the cytoplasm is optically heterogeneous.

Fig. 8. Cells (24-hr) in agar containing rivanol 1:6000. Dark-field contrast. The loose, poorly delineated cell membrane, with outpouchings and rips through which large chromatin granules are extruded, is seen. Several granules are scattered within the cells.

The lag-phase of growth was significantly prolonged, the growth process deranged, but cell division was less disturbed and continued until the cells stopped growing. A second phase then appeared: the manifestation of structural-morphological changes in the cytoplasm and cell membrane. Sublimate, in contrast to the other disinfectants, evoked a much more acute destruction of cell life, as confirmed by electron microscopic studies (Milyavskaya and Kavenoka, 1947).

Studies of the effects of rivanol [2-ethoxy-6, 9-diaminoacridine lactate], hydrogen peroxide, hexachlorophene and heptylresorcinol were carried out by the same methods, but because of the length of this article we will only mention that the effects of these disinfectants were to prolong the lag-phase of growth, to inhibit or disturb the first stage of cell division, and, consequently, to cause cessation of growth. The "active phase" which followed – a manifestation of cell destruction – was not well distinguished from the pathological processes evoked by other agents but, however, showed several peculiarities such as the differentiation of the chromatin in the presence of rivanol into more widely dispersed, clearly formed particles. The preparations we studied gave rise to cytoplasmic and membrane alterations analogous to those described above (Fig. 8).

SUMMARY

1. The mechanism by which disinfectants act upon the microbial cell is manifested in bactericidal concentrations by the rapid and abrupt derangement of cellular colloids, as a result of which one catches the effect of this action only as sharply expressed structural changes. With bacteriostatic concentrations a delay in growth and cell division processes occurs and the cells remain unchanged for a long time, only the greatly sensitive ones among them being changed as by bactericidal action. In sub-bacteriostatic concentrations the following are observed: lengthening of the lag-phase, delay in the processes of cell division, derangement of processes underlying cell growth and pathological structural-morphological changes.

2. The degree to which the degenerative structural-morphological changes are manifested depends both on the nature and concentration of the chemical agent and on the sensitivity of the bacterial cell.

3. We may consider that the processes of cellular degeneration proceed uniformly and have a common character.

4. The phase-contrast method and especially the dark-field contrast method in combination with observations of culture growth in the Peshkov chamber may be used for the rapid determination of bactericidal and bacteriostatic doses of disinfectants.

5. Dark-field microscopy reveals the finest structural changes in the cytoplasm and cell membrane, which permits certain details of the mechanism by which disinfectants act to be brought to light.

LITERATURE CITED

1. V. Ya. Aleksandrov, 1948. Soveshch. po belku. Izd-vo AN SSSR, p. 95.
2. S. I. D'yakov, A. N. Ivanova and A. M. Malchenkov, 1939. ZhMEI, No. 12.
3. A. M. Malchenkov and S. I. D'yakov, 1957. Laboratornoe delo, No. 4.
4. P. F. Milyavska a, 1951. Tr. Tsentr. n.-i. dezinfekts. in-ta (TsNIDI), No. 7.
5. P. F. Milyavskaya and F. Ya. Kavenoka, 1947. TsNIDI, No. 3.
6. D. N. Nasonov and V. Ya. Aleksandrov, 1940. The Reactions of Living Matter to External Stimulation [in Russian] Medgiz.
7. M. A. Peshkov, 1955. Usp. Sovr. biol. 39, No. 2.
8. B. I. Rukavtsev, 1958. Zh. MÉI, No. 5.
9. K. O. Frolich, 1955. Phasenkontrastmikroskopie in der Medizin. Jena.
10. A. Wilsca, 1953. Ann Med. exptl. et biol. 31, N 2, Helsinki.
11. A. Wilsca, 1954. Mikrosk. Ztbl. mikrosk. Forsch. u. Methodik 9, H. 1-4, I.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

PHYSIOLOGICAL DIFFERENCES BETWEEN STRAINS OF ROOT NODULE BACTERIA OF PEA AND VETCH DIFFERING IN NITROGEN-FIXING ACTIVITY

M. V. Fedorov* and L. Nitsé

K. A. Timiryazev Moscow Academy of Agriculture
Translated from Mikrobiogiya, Vol. 30, No. 3,
pp. 473-477, May-June, 1961
Original article submitted June 7, 1960

In our preceding communication (Fedorov and Laslo, 1956), data were presented concerning the fact that the root nodule bacteria of pea and vetch isolated from nodules at different stages of development of the plants have different nitrogen-fixing activity. Similar data have been reported by Lopatina (1951), Lazareva (1951), Berezova and Rempe (1952), Fedorov and Glavachkova (1956), Thornton et al. (Chen, Nicol, and Thornton, 1940; Thornton, 1945), Nutman (1946), and others. However, they either did not carry out a more detailed physiological characterization of such strains, or data were given on a very limited scale. It is for this reason that we undertook the present investigation in which we studied the physiological properties of strains of root nodule bacteria isolated from nodules that were obtained by means of inoculation of peas and vetch with original strains of root nodule bacteria No. 248 (pea) and 134 (vetch). Pure cultures of these bacteria were isolated from the nodules during the phase of blossoming and small bean formation. In subsequent experiments, they were again used for inoculation along with the original strains. Experiments showed that the strains obtained in this manner have a higher nitrogen-fixing activity. Strain No. 13 (for peas) and strain No. 32 and No. 33 (for vetch) were isolated from the nodules of plants grown on a background of 0.1 and 0.5 norms of nitrogen using Hellriegel's mixture in sand culture.

The utilization of these strains for inoculation showed that under the influence of strain No. 13, which was isolated during the blossoming period, pea plants accumulated one and one-half times more nitrogen than plants inoculated with original strain No. 248. In exactly the same way, vetch plants inoculated with strain No. 32 accumulated 20% more nitrogen. But those inoculated with strain No. 33 that was isolated from nodules during the ripening phase gave less nitrogen than those inoculated with original strain No. 134.

We thus had at our disposal two more active strains and one less active one; these were the strains that were tested along with original strains No. 248 and 134, from which they had been obtained. The physiological properties of these strains were studied after their preliminary cultivation in the laboratory over a period of two years.

In order to establish the nature of the physiological differences between them, we determined the productivity of the synthesis of cellular material by them during the utilization of bound forms of nitrogen, and the activity of respiratory enzymes (dehydrogenases and oxidases). The experiments on the determination of the productivity of the synthesis of cellular material were set up on a chemically-defined medium containing various carbon and nitrogen sources. Several carbohydrates, alcohols, and organic acids were used as carbon sources. Their concentration in the medium was 0.1 M. Sodium nitrate and ammonium chloride were used as nitrogen sources. They were added to the medium in amounts to give a ratio of 20:1 between the carbon of the energy-yielding substance and nitrogen. Sorensen's phosphate buffer was added to the medium in order to maintain a constant pH. The experiments were set up in flasks containing 500 ml of nutrient medium. Sterilization was carried out in an autoclave at 0.5 atm pressure for 30 min. Two ml of bacterial suspension of the appropriate strain was added to each flask of medium; the suspensions contained equal numbers of cells of the test bacterium. Incubation was carried out at 28 C for 30 days.

At the end of the experiment, the bacterial cells that had grown were separated from the nutrient medium by centrifugation at 3500 rpm. The bacterial mass obtained was dried and weighed, and then its content of total, protein, and nonprotein nitrogen was determined (Table 1).

The numerical material in Table 1 shows that the strains of root nodule bacteria having the higher nitrogen-fixing activity in nodules were also more active under conditions of laboratory culture. They synthesized more cellular material per unit of energy-yielding material on various carbon and nitrogen sources. In addition to this, they contained more protein nitrogen in the cellular material itself. This peculiarity enables us to

* Deceased.

TABLE 1. Productivity of the Synthesis of Cellular Material in the Root Nodule Bacteria of Pea and Vetch during Growth on Various Carbon and Nitrogen Sources (average data of two repetitions)

Strains	Nitrogen source $NaNO_3$				Nitrogen source NH_4Cl			
	bacterial dry mass, mg/g C source consumed	protein and nonprotein N content, mg/g bacterial mass			bacterial dry mass, mg/g C source consumed	protein and nonprotein N content, mg/g bacterial mass		
		protein N	nonprotein N	protein to nonprotein N ratio		protein N	nonprotein N	protein to nonprotein N ratio
Sucrose								
248	12.8	14.43	2.12	6.80	12.1	13.38	2.34	5.74
13	14.5	15.25	1.97	7.77	13.5	14.84	2.16	6.85
32	15.6	13.39	1.81	7.40	12.9	13.81	2.11	6.86
33	13.7	11.67	1.74	6.72	11.5	12.88	2.28	5.67
134	14.5	13.18	1.82	7.24	11.1	13.37	2.16	6.20
Glucose								
248	12.6	13.14	1.96	6.72	11.6	12.48	2.14	5.80
13	14.1	13.99	1.89	7.44	13.0	13.32	2.00	6.67
32	14.6	11.80	1.70	6.97	12.3	12.35	1.85	6.70
33	11.8	11.12	1.68	6.62	10.7	11.76	1.96	6.00
134	13.5	11.33	1.67	6.80	11.8	12.08	1.92	6.30
Maltose								
248	11.2	8.88	1.34	6.63	10.7	8.52	1.68	5.00
13	12.8	10.07	1.43	7.02	11.6	9.63	1.67	5.77
32	12.3	10.55	1.55	6.80	11.8	8.77	1.53	5.76
33	10.6	7.42	1.44	5.85	10.2	7.62	1.58	4.84
134	11.0	8.70	1.40	6.24	11.3	8.36	1.64	5.10
Galactose								
248	11.7	11.51	1.69	6.80	10.8	10.74	1.93	5.67
13	13.6	12.80	1.82	7.20	12.3	12.27	1.94	6.32
32	12.8	11.54	1.66	6.94	12.8	11.82	1.93	6.14
33	11.3	10.78	1.72	6.28	10.5	10.38	2.00	5.20
134	11.7	11.01	1.69	6.52	11.4	11.11	1.99	5.60

assume that, in strains of root nodule bacteria which fix atmospheric nitrogen vigorously in nodules, the enzyme systems participating in the oxidation of energy-yielding material are more active, and with the aid of these enzymes, they utilize the biologically-useful energy released during the course of oxidative processes more completely.

However, they not only synthesize more cellular material, but its content of protein nitrogen is greater as well.

In active strains, the cellular protoplasm is evidently in a more active state because a large amount of protein substance participates in the formation of its structure. It is this protoplasmic structure, which contains a full complement of essential enzymes, that enables them to carry out vital processes not only at a high rate, but with the more efficient utilization of the chemical energy of the oxidation of energy-yielding material as well. When they utilize the carbon source more efficiently, an additional requirement for nitrogen arises in them, and they meet this requirement by increasing the rate of nitrogen fixation. Since all of this is closely associated with the activity of respiratory enzymes, in the next experiments we studied the dehydrogenase and oxidase activities of these same strains of pea and vetch root nodule bacteria.

The first experiment was set up to determine the activity of dehydrogenases.

The bacteria for this experiment were grown on a chemically-defined agar medium with glucose, and were washed off with sterile water; suspensions containing a known number of bacterial cells (5 billion per 1 ml) were prepared from these washes. One-ml amounts of these suspensions were placed in Thunberg tubes to which 20 ml of a hydrogen donor solution in a concentration of 0.1 M was added, as well as 2 ml of Sorensen's phosphate buffer at pH 6.94. Methylene blue (1 ml of a 0.1 % solution) was added to the tube as a hydrogen acceptor. After this, air was pumped out of the tubes, and they were placed in an incubator at 28 C. Next, the start and end of the decolorization of methylene blue was carefully observed.

The experiment performed in this manner showed that the dehydrogenase activities of the different strains

TABLE 2. Dehydrogenase Activity of Pea and Vetch Root Nodule Bacteria (The end of methylene blue reduction is given in hours. Average data of two repetitions)

Hydrogen donors	Strains of root nodule bacteria				
	248	13	32	33	134
Glucose	4	3	1.5	4	2.5
Maltose	11	8	8	11	10
Lactose	4	3	2	4	3
Mannitol	6	4	5	8	6
Galactose	7	6	5	9	7
Glycerol	9	6	6	10	8
Lactic acid	11	8	7	14	9
Citric acid	25	20	16	24	19
Succinic acid	12	8	7	12	10
Malic acid	8	6	6	10	8
Tartaric acid	12	8	6	14	10
Acetic acid	14	10	10	16	12

TABLE 3. Activity of Molecular Oxygen Consumption in Various Strains of Root Nodule Bacteria (average data of two repetitions)

Strains		Molecular oxygen consumed in 4 hr of experiment, in mm^3		
		In individual vessels	Average	In % of original strains
Pea bacteria	13	87.7 89.4	88.6	247.0
	248	32.4 38.6	35.5	100.0
Vetch bacteria	32	95.3 99.9	97.6	175.0
	33	42.3 42.7	42.5	76.6
	134	54.0 57.0	55.5	100.0

of root nodule bacteria which we studied are not the same (Table 2).

These data show that, in the strains of root nodule bacteria which had the higher nitrogen-fixing activity in nodules, the dehydrogenase activity was also considerably higher, whereas in the strains with the lower nitrogen-fixing activity,it was considerably lower. We had noted this type of phenomenon in other cultrues of root nodule bacteria (Fedorov and Laslo, 1956; Fedorov and Egorova, 1958; Fedorov and Glavachkova, 1956). It was completely confirmed with these strains as well. It was also curious to note that different hydrogen donors gave practically monotypic results. Since the majority of them is dehydrogenated by specific dehydrogenases, it can be regarded as established that various dehydrogenases are in a more active state in the cells of the more active nitrogen-fixers. Therefore, the mobilization of hydrogen in them will occur more rapidly during the utilization of quite different carbon sources. The parallelism between nitrogen-fixing activity and dehydrogenase activity enables one to make a preliminary evaluation of the corresponding strains of root nodule bacteria by a more rapid method. Instead of setting up greenhouse experiments and subsequently determining the atmospheric nitrogen fixed, a rapid determination of dehydrogenase activity can be made and a preliminary conclusion can be given in order to select the most active strains.

This method is of considerable interest, especially in combination with the determination of the productivity of the synthesis of cellular material. The parallelism between these indicators is evidently connected with the fact that active forms of hydrogen, mobilized by dehydrogenases from the material being oxidized, are necessary not only for the production of biologically useful energy for the synthesis of cellular material, but also for the reduction of the primary products of nitrogen fixation to amino acids and proteins. The more rapid the hydrogen mobilization, the more molecular nitrogen apparently undergoes reduction and is incorporated not only in the synthetic processes of the bacterial cells themselves, but is also secreted into the nodule cells and is further utilized by the plant.

The second experiment was set up to determine the activity of molecular oxygen consumption by the cells of the root nodule bacteria under investigation.

The bacteria for this experiment were also grown on defined agar medium with sodium nitrate. The cells which had grown were washed off the agar with sterile tap water, and suspensions containing equal numbers of cells were prepared from the washings of each bacterial strain. Suspensions containing 5 billion cells per 1 ml were prepared for the experiment. Next, 2 ml of this bacterial suspension and 3 ml of a glucose solution in a concentration of 0.1 M were added to each Warburg vessel. The Warburg experiment was continued for 4 hr at 30 C (Table 3).

These data also show that the strains of pea and vetch root nodule bacteria having the highest nitrogen-fixing activity in nodules consumed molecular oxygen more actively as well. In this respect, the oxidases behaved similarly to the dehydrogenases. All of the enzyme systems in the more active strains are apparently in a more active state, and the vital processes of these cells go on at a higher level. However, since they utilize carbon sources more efficiently for the synthesis of cellular material, it must be assumed that the basis for their higher nitrogen-fixing activity is associated with the enzyme systems

participating in the release and further utilization of biologically useful energy. Apparently, in the cellular protoplasm of the active fixers of molecular nitrogen, the entire enzyme system works more harmoniously.

Thus, those strains of root nodule bacteria that fix molecular nitrogen more actively in nodules under laboratory conditions differ from the less active strains not only by their more efficient utilization of the carbon source for the synthesis of cellular material, but also by a higher dehydrogenase and oxidase activity. Since these physiological properties can be determined easily and rapidly, it is convenient to make use of the above-mentioned indicators in making a preliminary evaluation of the activity of strains of these bacteria. It is necessary to set up greenhouse experiments with the precise determination of the actual fixation of atmospheric nitrogen only for purposes of a final evaluation of their nitrogen-fixing activity.

SUMMARY

1. Strains of pea and vetch root nodule bacteria having different nitrogen-fixing activities in the nodules of these plants differ from one another in a number of physiological characteristics, which are easily determined in laboratory cultures of these bacteria.

2. The strains that fix molecular nitrogen actively in nodules synthesize their cellular material more efficiently on various carbon and nitrogen sources. Not only is more cellular material synthesized by them per unit of energy-yielding substance consumed, but it has a higher protein content as well. Because of this, their cellular protoplasm becomes more active, and the enzymes which it contains oxidize energy-yielding material more rapidly and more effectively utilize for synthesis the biologically useful energy released in the course of oxidative processes.

3. The parallelism between the activity of molecular nitrogen fixation in nodules and oxidase and dehydrogenase activity in the cells of pea and vetch root nodule bacteria makes it possible to use the latter indicators, together with the determination of the productivity of the synthesis of cellular material, for a preliminary evaluation of their nitrogen-fixing activity. A final evaluation of this, however, must be made by means of greenhouse experiments accompanied by a precise determination of the amount of atmospheric nitrogen fixed by them.

LITERATURE CITED

1. E. F. Berezova and E. Kh. Rempe. 1951. Mikrobiologiya 21, No. 4.
2. N. M. Lazareva, 1951. Tr. Vses. in-ta s.-kh. mikrobiol. 12.
3. G. V. Lopatina, Tr. Vses. in-ta s.-kh. mikrobiol. 12 (1951).
4. M. V. Fedorov and D. Laslo, Izv. Timiryazevsk. s-kh. akad., No. 2 (1956).
5. M. V. Fedorov and E. Glavachkova, Izv. Timiryazevsk. s.-kh. akad., No. 1, 51 (1956).
6. M. V. Fedorov and S. V. Egorova, Mikrobiologiya 27, No. 5, 593 (1958).
7. H. Chen, H. Nicol and H. Thornton. 1940. Proc. Roy. Soc. London, Ser. B, No. 257.
8. H. Thornton. 1945. Nature 156, 654.
9. P. Nutman. 1946. J. Bact. 51, 411.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

ADJUSTMENT OF VARIOUS AZOTOBACTER STRAINS ON THE ROOTS AND PHIZOSPHERE OF SPRING WHEAT GROWN ON A PODZOL SOIL

L. I. Vigorov

Institute of Forestry, Sverdlovsk
Translated from Mikrobiologiya, Vol. 30, No. 3, pp. 478-483, May-June, 1961
Original article submitted June 27, 1960

The unsatisfactory results of the use of azotobacterin in the cultivation of plants on podzol soils have been repeatedly noted.

It is known that, aside from soil toxicity, the conditions of the plant rhizosphere have the greatest effect on the reproduction of azotobacter on roots. The root formation of wheat has been noted as having both a positive [Sheloumova and Shtutser, 1947] and a depressing action on azotobacter [Krasil'nikov, 1934, 1944; Sushkina, 1949]. The complex interrelationship between azotobacter and the rhizosphere bacteria can also affect its adjustment in the rhizosphere [Van-Tszy-fan, 1958]. It is possible to increase the adjustment of azotobacter in the rhizosphere of wheat in several ways, in particular by changing the soil reaction through liming [Vigorov, 1958] or through the use of organic fertilizer [Gebhardt, 1953; Gel'tser, Poperekova, 1955]. Because of its frequent growth on wheat roots, data on the selection of azotobacter strains adapted to wheat rhizosphere conditions are known [Zinov'eva, 1958, 1960]. The existence of acid-fast species [Azotobacter indicum, Fedorov, 1952] and strains of azotobacter [Blinkov, 1955; Bychkovskaya and Shklyar, 1959] would make one assume them more easily adaptable in the wheat rhizosphere, where formation in the roots of organic acids may be one reason for the toxicity of rhizosphere soils for azotobacter. The study of the adjustment of the above-mentioned forms of azotobacter on wheat grown on a podzol soil has significant interest.

In the period 1958-1959 we studied the possibility of increasing the adjustment of azotobacter on wheat roots by the use of acid-fast strains isolated by Blinkov (strain 1a and 41a) and strain 28 selected by Zinov'eva for wheat. Cultures of bacteria were obtained directly from the authors shortly before planting. * As a control a local, moderately pigmented strain of azotobacter, ULTI-1, isolated from the dark-colored turf-podzol soil of the experimental land where the wheat had been planted during the first year of the experiment, was used. The unsatisfactory adjustment of the non-adaptive local strains of azotobacter in the rhizosphere of spring wheat grown on podzol soils without liming or the introduction of organic fertilizer was shown in the foregoing works [Vigorov, 1958; Tribunskaya, 1954].

The Adjustment of Acid-Fast Strains of Azotobacter (Experiments of 1958)

Grain of Diamant spring wheat was purposely infected with a large number of bacteria (10 g of bacterial slime + 25 g turf per 100 g grain). Sowing was done in a plot with dark-colored turf-podzol soil typical for the Middle Urals, lacking in lime to the amount of 12 tons/ha by hydrolytic acidity. The plots where the grain infected with acid-fast strains of azotobacter was sown were not limed, since the goal was to ascertain the possibility of the adjustment of these bacteria in the rhizosphere under the most unfavorable conditions. The section of land where a strain of local azotobacter-infected grain was sown was limed for testing the data of the previous year concerning the positive effect of liming on the adjustment of local azotobacter on the wheat roots; part of the land was not limed. Half-carbonized lime in amounts of $^1/_2$ and 1 normal by hydrolytic acidity was used. Determination of the number of azotobacter cells and zoogloea in the wheat rhizosphere was made by coating its roots (freed only slightly from the soil) with an agarized Ashby medium with sucrose. Earlier [Vigorov, 1958] it was shown that the use of unwashed roots avoided exaggerated data and provided more exact facts on the extent of azotobacter colonization of the wheat rhizosphere.

In Table 1 data are presented on the number of wheat root azotobacter at various stages in the growth and development of the plant. For a more graphic demonstration of the number of azotobacter, its colonies on all roots of the individual plant were taken into consideration.

The data presented show that neither strain of acid-fast azotobacter is adapted to the roots of the type of wheat studied, cultivated in turf-podzol soil. Therefore it is not the acidity but other adverse factors which determine the destruction of azotobacter in the rhizosphere of wheat, and these strains, adapted to soil acidity, active aluminum and other conditions of acid soils, are not adapted to the complex of adverse conditions going into the bacterial condition of wheat.

* We take this opportunity to thank the above-mentioned investigators for donating the cultures.

TABLE 1. Adjustment of Acid-Fast and Local Strains of Azotobacter in the Rhizosphere of Wheat. [The number of azotobacter on the roots of a single plant: the average for six plants; 1) the primary roots; 2) the secondary root]

Experimental conditions	Growth stage and analysis date					
	end of 2nd leaf growth (May 23)	end of tillering (June 11)	blooming (July 10)		ripening, maturity (August 10)	
	1	1+2	1	2	1	2
Control plants without bacterial infection and liming	0	0	2	1	0	0
Strain 1a, without lime	0	7	7	8	0	0
Strain 41a, without lime	0	9	7	2	1	0
ULTI-1, without lime	0	1	0	2	0	0
ULTI-1, limed 1/2 hydrolytic acidity	42	Continuous root overgrowth	24	140	9	30
ULTI-1, limed 1 hydrolytic acidity	56	" "	62	91	9	60

It is characteristic that a single colony of acid-fast azotobacter appearing around the roots of wheat in Ashby agar has small size and shows slow growth in comparison with local azotobacter. The diameter of their colonies after five days is 3-4 mm, whereas in the same time a colony of the local strain grows to 1-2 cm. The introduction of lime aids the adjustment of a large number of local-strain azotobacter in the wheat rhizosphere, as was shown in the experiments of the previous year. With an increase in the amount of lime to the full norm of hydrolytic acidity the number of bacteria on the roots increases insignificantly in comparison with liming to half the norm. In plants growing in limed soil, azotobacter hold on in the rhizosphere of the secondary roots until the stage of green ripeness of the grain, but only single cells are retained by this time on the primary roots. At the same time, liming in the year lime was first used did not show an effect on the increase of azotobacter cells in the soil beyond the rhizosphere to such an extent that it could substantially alter the nitrogen content of the soil. In the August 10th distribution of 100 one g soil samples in Ashby agar no azotobacter were found in non-limed soil, 16 cells were found in that limed to $^1/_2$ the norm of hydrolytic acidity, and in that limed to the full norm – 65 cells per one g of damp soil. Tests on September 10th gave, per g of soil, correspondingly: 0-70-20 azotobacter cells. For comparison it is sufficient to point out that the number of azotobacter cells in one g of chernozem soil reached 3,000 and more. Minute fragments of lime extracted from the soil and placed in an elective agar together with soil fragments do not become overgrown with azotobacter colonies and the soil around these clusters is therefore not within the zone of azotobacter reproduction.

For clarification of the environmental condition of azotobacter in the rhizosphere of wheat in comparison with the soil beyond the sphere of influence of the roots, the reaction of root-bottom and extra-rhizosphere soil was studied. Tests were made with a thick suspension made by immersing the root system in water and gently separating it from the soil and soil covering. The roots of several plants not separated from the above-ground organs were immersed in a small amount of distilled water (pH 5.5-5.6). From 1.5-2 min after immersing the roots and careful stirring of the suspension the concentration of hydrogen ions was determined. At the same time the reaction of a suspension of soil from between planted rows was studied, with the proportion of soil to water – 1:2. The data on the pH value of the inter-row and root-bottom soil of Diamant wheat is shown in Table 2.

In the tillering phase the primary roots of the plant receiving lime (in contrast to the roots of the control plants) were surrounded by a thick root-bottom soil covering with a large number of $CaCO_3$ fragments in which fine roots grew. The reaction of the suspension of root-bottom soil from plants in limed plots was inert or close to it in contrast to the inter-row soil, the neutralization of which, because of the lime used, proceeded slowly. In this way the adjustment of azotobacter in the wheat rhizosphere of limed soils was explained. In evaluating the data on the reaction of the wheat rhizosphere medium we must also note that the pH value of the thick viscous soil suspensions did not correlate completely with the actual active acidity value of the undiluted natural soil solution, though it was closer to it than that of the soil extracts. The great contrast between the pH of the soil suspensions and the soil extracts has already been made long ago [Gedroits, 1923].

TABLE 2. Reaction of the Environment (pH) in the Rhizosphere of Wheat Grown on a Dark-Colored Turf-Podzol Soil. [1) pH of the suspension of inter-row soil; 2) pH of the suspension of root-bottom soil]

Experimental variant	Tillering (Feb. 7)		Blooming (July 10)	
	1	2	1	2
Control plants	5.3	5.2	5.8	4.2
Limed plants (1/2 hydrolytic acidity)	5.6	6.9	6.2	7.0
Limed plants (1 hydrolytic acidity)	6.2	7.0	6.6	7.3

Study of the Adjustment of Wheat-Adapted Azotobacter on Roots (Experiments of 1959)

In the given series of experiments the strains of Ural azotobacter used in the preceding experiments and wheat-adapted strain 28 of the Ukrainian Institute of Microbiology were compared. Inoculation of two types of wheat grain — Diamant and Moscow — was made with a suspension of azotobacter with one g bacterial substance to 25 g grain. In contrast to the previous year's experiment the plants were grown in a less fertile gray podzol soil poor in humus (1.5% humus) obtained from a pine forest. The lime requirements of the soil were 8.2 tons/ha by hydrolytic acidity. The active acidity of water extracts (1:5) — 5.0-5.3 pH. In part of the tests plants were grown in limed soil. In this case completely carbonized lime was used in amounts of $^1/_2$ normal by hydrolytic acidity.

The data shown in Table 3 on the adjustment of azotobacter in the rhizosphere of wheat give the average number in 6-8 counts. Determinations were made not only for the presence of azotobacter in root-bottom soil, but also for their presence immediately on the root surface and on washed roots. The data on the number of azotobacter cells on washed roots is sufficiently typical in view of the small space between colonies and the difficulty of calculation due to continuous root overgrowth by bacterial masses. Determinations of the number of azotobacter cells in the rhizosphere of soft wheat showed a sudden contrast in adjustment between the adapted and local strains used in favor of the wheat-adapted strains. The number of colonies of adapted strains on the wheat roots was 10-20 times greater than in the case of non-adapted strains. Azotobacter colonies of adapted strain 28, growing around the roots in a dense mass, were dry and wrinkled, whereas those of the Ural strain were slimy and wrinkled. The introduction of lime made a difference only for the adapted strains, increasing the number of rhizosphere colonies by 1.5-2 times.

Comparison of the data on the adjustment of local azotobacter strains (ULTI-1) on the roots of one type of wheat (Diamant) for the two years indicated that in 1959 (in contrast to 1958) this strain did not show an increase in adjustment in the wheat rhizosphere in limed soil. This may be primarily explained by the use in the test of strains isolated from the same dark-colored turf-podzol soil (obtained from a birch forest) on which the 1958 test was conducted, when a positive effect of liming on azotobacter adjustment in the wheat rhizosphere was also shown. It should be borne in mind that the great difference in chemical characteristics of the soils used in relation to the humus content and active aluminum and the fact that half-carbonized lime was used in 1958 led to a more rapid neutralization of root-bottom soils, whereas completely carbonized lime, dictating a slow neutralization reaction, was used in 1959. Both years differed in climatic conditions — the 1958 growing period was hot and dry, but in 1959 it was rainy and cold.

One should note that even when adapted azotobacter were used there were not more than 20-30 azotobacter colonies on the roots of an individual mature wheat plant. The greatest number of azotobacter counted in the 1958 tests amounted to 50-140 colonies for the local strains on plants grown on limed ground. Even if we assume that azotobacter are present in the rhizosphere of plants in the form of zoogloea rather than primary cells, the total mass of this type of bacteria is so insignificant in comparison with the root mass of a mature plant that it is difficult to expect them to have a strong influence on nitrogen-feeding plants after the tillering period. Zoogloea does not show up in a microscopic examination of the surface of wheat roots grown from azotobacter-treated grain, and just how great the accumulation of cells on the wheat root surface is can not be established. Our attempts to discover additional sources of nitrogen-feeding wheat sporelings living on the roots at the expense of azotobacter by analysis of guttulate fluid [Vigorov, 1954] have not given positive results. Therefore, the improvement of nitrogen-feeding of wheat plants under the influence of the azotobacter in the rhizosphere mentioned in the literature [Gebhardt, 1958] deserves additional investigation.

SUMMARY

1. Acid-fast strains of azotobacter do not adjust to any observable extent in the rhizosphere of spring wheat grown on a dark-colored turf-podzol soil. In this respect they do not differ appreciably from local Middle Ural azotobacter strains.

2. Wheat-adapted azotobacter (strain 28) adjusts well on wheat roots growing on podzol soil without liming and is still present in the rhizosphere during the

TABLE 3. Adjustment of Local and Adapted Azotobacter on the Roots of Soft Wheat Grown on a Gray Podzol Soil. (The number of colonies of azotobacter on one plant; 1) primary roots; 2) secondary roots)

Experimental variant	End of tillering - start of yield (July 1)				Plant blooming and shedding (July 24)			
	Diamant		Moscow		Diamant		Moscow	
	1	2	1	2	1	2	1	2
Unwashed roots								
Without azotobacter, without $CaCO_3$	0	0	0	0	0	0	1	0
" + $CaCO_3$	0	0	0	0	0	0	2	0
ULTI azotobacter, without $CaCO_3$	1	2	1	0	0	1	0	0
" + $CaCO_3$	2	3	1	2	2	1	0	0
Strain 28, without $CaCO_3$	13	14	12	19	4	3	1	2
" " + $CaCO_3$	24	31	21	22	9	18	6	8
Washed roots								
Without azotobacter, without $CaCO_3$	0	0	0	1				
" + $CaCO_3$	1	0	1	1				
ULTI azotobacter, without $CaCO_3$	1	3	2	6				
" + $CaCO_3$	1	2	3	7				
Strain 28, without $CaCO_3$	6	15	12	22				
" " + $CaCO_3$	Continuous overgrowth			32				

plant's blooming period. The introduction of carbonized lime increased the number of strain 28 cells in the rhizosphere by 2-6 times, depending on the type of root and the stage of growth.

3. Limed soil sharply increases the adjustment of local azotobacter strains in the rhizosphere of soft wheat grown on a turf-podzol soil.

LITERATURE CITED

1. G. N. Blinkov. 1955. Mikrobiologiya 24: 415.
2. A. L. Bychkovskaya and M. Z. Shklyar. 1959. Mikrobiologiya 28: 336.
3. Van Tszy-fan. 1958. Doklady TSKhA, 34, 94.
4. L. I. Vigorov. 1954. Priroda, 2: 106.
5. L. I. Vigorov. 1958. Mikrobiologiya 27: 89.
6. L. S. Gebhardt, Collection: Problems on the Use of Bacterial Fertilizers [in Russian] (Kiev, 1953).
7. A. G. Gebhardt, Nauchn. Doklady Vyssh. Shk., Biol. Nauki, 3, 160 (1958).
8. K. K. Gedroits, The Chemical Analysis of Soils [in Russian] (1923).
9. F. Yu. Gel'tser and L. M. Poperekova, Agrobiologiya 3, 70 (1955).
10. Kh. G. Zinov'eva, Mikrobiologiya 27, 75 (1958).
11. Kh. G. Zinov'eva, The Interrelationship of Azotobacter and the Higher Plants [in Russian] (Kiev, 1960).
12. N. A. Krasil'nikov, Mikrobiologiya 3, 3 (1934).
13. N. A. Krasil'nikov, Mikrobiologiya 13, 187 (1944).
14. N. A. Sushkina, The Ecological-Geographical Distribution of Azotobacter in the Soils of the USSR [in Russian] (Izd-vo AN SSSR, 1949).
15. A. Ya. Tribunskaya, Mikrobiologiya, 23, 283 (1954)
16. M. V. Fedorov, The Biological Fixation of Atmospheric Nitrogen [in Russian] (Sel'khozgiz., 1952).
17. A. M. Sheloumova and Yu. M. Shtutser, Sov. Agronomiya 5, 72 (1947).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

RESISTANCE OF SUGAR BEET RHIZOSPHERE BACTERIA TO ANTIBIOTICS

P. Gyurkó, M. Kecskés, and E. Manninger

Laboratory of Soil Biology, Academy of Sciences, Sopron, Hungary

Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 484-488, May-June, 1961
Original article submitted August 6, 1960

It is known that the microflora of a root-forming zone contrast quantitatively and qualitatively with the microflora of rootless soil [Krasil'nikov, 1958; Voznyakovskaya and Zhil'tsova, 1958; Kecskés, 1960; Feher, 1954; Federov, 1951]. The root-bottom zone does not have sharp boundaries and is not fixed. It changes in relation to the physical and biological conditions predominant in the soil, and, in the first place, in relation to the degree of root formation. Inside this zone the effect of root formation decreases according to the degree of root removal [Macura, 1958 and others]. The bacteria living in the rhizosphere, specifically at the edge of the zone, are, it seems, stationary and exposed to the "aggressive" action of other soil microorganisms. From the time of planting until the formation of the root system the rhizosphere bacteria may survive in the soil without the safeguard of root formation. At this point in the struggle with other soil microorganisms the production of antibiotics and their resistance to those antibiotics produced by other microorganisms is of great significance to the rhizosphere bacteria.

The problem of the sensitivity and resistance of rhizosphere bacteria to antibiotics is the topic of our study.

METHOD

For the experimental study we used rhizosphere bacteria formed from the roots of six-week-old sugar beets by the Tepper method [cited by Fedorov, 1952]. From the dish in which a paste from ground-up roots was placed, 42 strains of bacteria were isolated. In the process of the study some of these strains proved to be identical.

The characteristics of these strains are shown in Table 1.

Since it is not possible to reproduce the natural conditions prevailing in the soil and rhizosphere, we used purified antibiotics of diverse antibacterial spectra in the tests in order to produce detailed data on the sensitivity and resistivity of rhizosphere bacteria. The following antibiotics were used in the tests: penicillin (Egyesult Györgyszer és Tápszergyar, Budapest), streptomycin (dihydrostreptomycin és Tápszergyar, Budapest), vionactan (viomycin-pantothenatsulfate, "CIBA"), chloramphenicol (Györgyszeripari Kutató Intézet, Budapest), oxytetracycline (HCl Chinoin, Budapest), canacine (canamycin-sulfate Continental Pharma, Brussels).

For the cultivation of rhizosphere bacteria the following media were used: MPA (meat extract – 10 g, peptone – 5 g, NaCl – 5 g, agar – 20 g, distilled water – 1 l, pH = 6.8), Chapek (sucrose – 30 g, $NaNO_3$ – 2 g, K_2HPO_4 – 1 g, KCl – 0.5 g, $MgSO_4$ – 0.5 g, $FeSO_4$ – 0.01 g, agar – 20 g, distilled water – 1 l, pH = 6.8).

The action of the antibiotics on the bacteria was studied in meat-peptone agar, in which all 16 of the

TABLE 1. Characteristics of Rhizosphere Bacteria

Strain No.	Form	Size, μ		Spores	Gram staining	Acid and alcohol fastness	Motility	Denitrification
		length	width					
1	Rod-shaped	1—1.8	0.5	—	—	+	+	+++
2	»	1—1.5	0.6—0.8	—	—	++	+	+++
3	»	1.2—1.4	0.7—0.9	—	—	++	+	+++
4	»	1,5—2.5	0.5	—	—	++	—	++
5	»	1.2—2.0	0,5	—	—	++	—	+++
6	»	1.2—1.5	0 7—0.8	—	—	+	+	+
7	Chain and rod-shaped	2.8—3.2	1.6—1.8	—	—	—	+	+
8	Rod-shaped	2.8—3.0	1.0—1.3	+	+	—	+	+
9	»	0.8—1.2	0.4	+	+	—	+	+
10	»	1.5—2.5	0.5—0.6	—	—	++	+	+
11	»	1.5—2.5	0.6—0.6	—	—	++	+	+++
12	»	1.5—2.5	0,5—0.6	—	—	+	+	+
13	Occasional small chains	1.8—2,0	0 5—0.7	—	—	+	+	+++
14	The same	1.2—2.0	0.6—0,8	—	—	+	+	+++
15	Rod-shaped	1,8—2.0	0.5—0.6	—	—	++	+	+
16	»	0,8—1.2	0.5—0.6	—	—	+	+	+++

TABLE 2. Effect of Various Concentrations of Antibiotics on the Growth of Rhizosphere Bacteria

Antibiotic	Antibiotic content in 1 ml substrate	Bacteria strain No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Penicillin, ME	2.0	+	+	+	+	−	+	−	−	−	+	+	+	+	+	−	+
	0.2	+	+	+	+	−+	+	−	+	−	+	+	+	+	+	+	+
	0,02	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+
	0,0002	+	+	+	+	+	+	−+	+	+	+	+	+	+	+	+	+
	Control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Streptomycin (dihydrostreptomycin), μg	100	−	−	−+	−	−	+	−	−	−	−+	−	−+	−	−	−	−+
	10	−	+	+	+	−	+	−	−	−	+	+	+	+	+	−	+
	1	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+
	0,1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	0,01	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vionactan (Viomycin-pantothenatsulfate), μg	50	+	−+	+	+	+	+	−	−	−	+	+	+	+	+	+	−+
	5	+	+	+	+	+	+	−+	+	−+	+	+	+	+	+	+	+
	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	0.05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	0.005	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chloramphenicol (chloromycetin), μg	50	−	−	−	−	−	−+	−	−	−	+	+	+	+	+	-	−
	5	+	−+	−+	+	+	+	−	−	−	+	+	+	−+	+	+	+
	0,5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	0.05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	0.005	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Terramycin (oxytetracycline HCl), μg	50	−	−	−	−	−	−+	−	−	−	−	−	−	−	−	−	−
	5	−	−	−	+	+	+	−	−	−	+	+	+	−+	+	−	−
	0.5	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+
	0,05	+	+	+	+	+	+	+	+	−+	+	+	+	+	+	+	+
	0,005	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Canacine (canamycin-sulfate), μg	50	−	−	−+	−	−	+	−	−	−	−	−	−	−	−	−	−
	5	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−
	0.5	+	−	+	+	+	+	−	−	−	−+	−+	−+	−	+	+	+
	0.05	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+
	0,005	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+
	Control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

* Note: + normal growth; -+ weak growth; - no growth.

strains examined grew well. The test-plate method proposed by Otte and Köhler [1958] was used with some modifications. Nine ml of culture medium and one ml of a water solution of various concentrations of antibiotics were put in Petri dishes with a 10 cm diameter, after which a suspension of the bacterium under consideration, in a physiological solution, was streaked on the surface of the congealed agar. The dishes were incubated in a thermostat at 21 C for 48 hr. At the same time control dishes free from antibiotics were used as controls of the growth of the strains. A solution in distilled water and a phosphate buffer pH = 6.3 was prepared from the antibiotics. After this the solution was sterilized by means of a bacterial filter and from it were prepared four dilutions from 10^{-1} to 10^{-4} in sterile condition. By the method described above one ml of the main solution and of its dilutions were transferred to the culture medium, resulting in the quantity of antibiotics per one ml shown in Table 2 for the appropriate culture media.

RESULTS

From the data shown in Table 2 it can be seen that rhizosphere bacteria, with the exception of three strains, showed sufficient resistance to antibiotics, since they grew even in the presence of large doses of antibiotics. A comparison of the data produced with species of Rhizobium [Herold, 1956] and soil chitin-decomposing microorganisms also showing considerable sensitivity to doses of specific antibiotics indicates the significance of the resistance of rhizosphere bacteria.

It is interesting that in spite of the well-known specificity of antibiotics many of the rhizosphere bacteria strains tested acted similarly toward a series of dilutions of different antibiotics.

Vionacta. chloramphenicol, oxytetracycline and canacine could e immediately compared since they were applied to the same cultures. In evaluating the effect of these antibiotics (Table 2) it is seen that if an antibiotic (for example, vionactan) did not affect more resistant strains No. 1-6 and 10-16, or if the effect was very weak, then the sensitive strains No. 7, 8, 9 were able to stand large doses of this antibiotic. In cases of antibiotics effective against resistant strains (for example, chloramphenicol and oxytetracycline) the sensitive strains stopped growing with insignificant doses of these antibiotics. Canacine showed the same antibiotic effects. The sensitive strains did not grow even when canacine doses of 0.005 μ g/ml were used, and in large doses it depressed the growth of nearly all resistant strains. In effectiveness against the rhizosphere bacteria under consideration, the antibiotics studied were arranged in the following order: canacine, oxytetracycline, streptomycin, chloramphenicol, penicillin, vionactan.

In this way the rhizosphere bacteria tested were divided into two groups: sensitive strains No. 7, 8, 9 and resistant strains No. 1-6, 10-16. From Table 1 it is seen that the sensitive strains differ from the resistant ones in size and some other characteristics. Two of them are gram-positive and form spores. Moreover, they are not acid-fast or alcohol-fast and are weak denitrifiers.

It remains to point out that among the 42 strains, isolated primarily from the rhizosphere of sugar beets, there occurred in only one strain sensitivity to the antibiotics tested. On the basis of this it may be assumed that they are rarely found in the rhizosphere. The resistant strains also showed a difference in resistance to antibiotics.

The greatest resistance produced in the presence of the same strong doses of antibiotics was shown by strain No. 6.

SUMMARY

1. The relationship of 16 strains of bacteria isolated from the rhizosphere of sugar beets to the following antibiotics was studied: penicillin, streptomycin, vionactan, chloramphenicol, oxytetracycline and canacine.

2. Only three strains showed sensitivity to antibiotics; the remaining 13 were resistant. In effectiveness of action the antibiotics studied were arranged in the following order: canacine, oxytetracycline, streptomycin, chloramphenicol, penicillin, vionactan.

LITERATURE CITED

1. N. A. Krasil'nikov, 1958. Soil Microorganisms and Higher Plants [in Russian] (Izd-vo AN SSSR, 1958).
2. Yu. M. Voznyakovskaya and G. K. Zhil'tsova. 1958. Mikrobiologiya 27 (1): 611.
3. D. Fehér. 1954. Talajbiológia (Akad. Kiadó, Budapest, 1954).
4. M. V. Fedorov. 1951. Mikrobiológia (Mezögazdasági Kiadó, Budapest, 1951).
5. M. V. Federov. 1952. Mikrobiologiai gyakorlatok (Mezögazdasagi Kiadó, Budapest, 1952).
6. M. Herold. 1956. Antibiotika (VEB Dsch. Verlag Wiss., Berlin, 1956).
7. M. Kecskés. 1960. Talajmikroorganizmusok antibiotikumérzékenysége. I. Néhány antibiotikum hatása a kitinbontó talajmikroorganizmusok növekedésére és kitinbontó tevékenységé." Agrókemia és Talajtan 9: 549.
8. M. Kecskés and E. Manninger Talajmikroorganizmusok antibiotikumérzékenysége. II. Különféle antibiotikumok hatása a Rhizobiumok növekedésére. Kézirat ban.
9. J. Macura. 1958. Folia Biol. Praha 4, 3, 129.
10. H. I. Otte and W. Köhler. 1958. Die Praxis Rezistenz- und Spiegel-bestimmungen zur antibiotischen Therapie (VEB C. Fischer Verlag, Jena, 1958).

EFFICACY OF PEAT–MANURE COMPOSTS WITH REFERENCE TO DEVELOPMENT OF BUTYRIC ACID BACTERIA IN THEM

S. S. Abyzov

All-Union Scientific Research Institute of Agricultural Microbiology, Leningrad
Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 489-493, May-June, 1961
Original article submitted December 3, 1960

One of the main tasks of agricultural science, including agricultural microbiology, is to obtain effective and cheap organic fertilizers and to discover the most efficient ways of applying them. An important way of increasing the efficacy of mineral and organic fertilizers is to apply them in combination.

In view of this the utilization of peat for the preparation of peat–manure composts containing added lime and phosphate fertilizers is of great practical importance. Despite the considerable literature dealing with the addition of lime and phosphates to peat composts, the nature of the action of these fertilizers on different groups of microorganisms, particularly butyric acid bacteria, has received little study so far.

The works of Berezova [1955], Berezova, Gusev, Rempe and Karpukhina [1955], Stepanova, Bylinkina and Figurina [1940], Pertseva [1950], Yung [1950], and others, have shown that the addition of lime and phosphate fertilizers to composts greatly enhances microbiological processes and results in the more rapid decomposition of organic matter. Yet this increases the loss of nitrogen and reduces the fertilizing value of the composts.

In this connection Lysenko [1953] has repeatedly pointed out that the nitrogen-fixing ability of certain microorganisms can be used to prevent any loss of nitrogen and even to enrich composts with it.

Investigations conducted in the All-Union Research Institute of Agricultural Microbiology have shown that this line of research can give positive results if suitable compost materials are chosen.

Azotobacter is often absent in peat–manure composts. Butyric acid bacteria, including the anaerobic nitrogen-fixer Clostridium pasteurianum, occur in large numbers and many authors regard them as the main nitrogen-fixers in composts.

Hence, a study of the effect of lime and phosphate fertilizers on the multiplication of butyric acid bacteria in peat–manure composts consisting of top or bottom peat and a discovery of the relation between bacterial numbers and the efficacy of composts applied to plants is of great interest.

For the experiments we used acid peats of different types: two bottom varieties (Pushkinskii and Romanovskii) and two top varieties (Malinovskii and Shcheglovskii). The peats differed from one another in degree of decomposition. The top peats were characterized by a microflora of the first stage of decomposition of organic remains, whereas the bottom peats were rich in a microflora typical of more advanced decomposition of plant remains. There were no azotobacter in the investigated peats.

Before the peats were composted they were well broken up and passed through a screen. Finely divided cow manure was added to the peat to make up 20% of the whole mass, which was thoroughly mixed. Mineral components (lime, rock phosphate meal, superphosphate) were added to the obtained mixture according to the experiment. The mixture was again thoroughly mixed and then put into wooden boxes of 16 kg capacity. The moisture content was maintained at 60% of the total water capacity and the composting temperature was about 18 C. Samples for microbiological and chemical analysis were taken every ten days. The butyric acid bacteria were counted in two media: in potato broth and in Winogradsky's nitrogen-free medium. Ashby medium was used to demonstrate azotobacter.

During 115 days of composting no azotobacter were found. Anaerobic nitrogen-fixers were found throughout the period of composting (Abyzov [1958]). Data on the effect of lime and phosphate fertilizers on the multiplication of butyric acid bacteria are given in Table 1. The results of the microbiological analyses show that lime and phosphate fertilizers had a much more stimulating effect on the multiplication of butyric acid bacteria in top-peat composts than in bottom-peat composts.

The addition of mineral fertilizers stimulated the multiplication of butyric acid bacteria so much that they became much more numerous in top-peat composts than in bottom-peat composts.

Both lime and rock phosphate meal increased the numbers of these microorganisms although the two groups of butyric acid bacteria (those counted in potato broth, and those of the Clostridium pasteurianum type counted in Winogradsky's nitrogen-free medium responded differently to the presence of lime and rock phosphate meal in the composts. High doses of lime were no more effective than low doses in increasing the multiplication of these microorganisms. On the contrary, in several cases small doses of lime had a greater effect on the multiplication of the investigated bacteria.

Combined addition of lime and rock phosphate meal in several cases did not enhance the multiplication of

TABLE 1. Effect of Lime, Rock Phosphate Meal, Superphosphate and Combinations of Them on Multiplication of Butyric Acid Bacteria during Composting of Peats (as % of control containing no added mineral components)

Microorganisms	Peats	2% L		10% L		5% RPM		2% SP		2% L + + 5% RPM		10% L + + 5% RPM		2% L + + 2% SP		10% L + + 2% SP	
		duration of composting, days															
		25	50	25	50	25	50	25	50	25	50	25	50	25	50	25	50
Butyric acid bacteria in potato broth	Bottom	10	4400	350	1000	550	240	550	520	550	1000	350	520	100	100	550	240
	Top	11 0000	21 700	110000	2200	110 000	41 700	500	100	110000	4170	110000	4200	110000	117 000	110 000	11 700
Clostridium pasteurianum type in Winogradsky's medium	Bottom	6	280	100	440	100	440	64	52	100	440	100	280	64	12	100	440
	Top	440	4000	440	2500	440	900	2	500	440	22 000	440	500	440	1200	440	5000

Note: Here and in Table 2: L) lime; RPM) rock phosphate meal; SP) superphosphate.

TABLE 2. Effect of Phosphate Fertilizers and Lime on Efficacy of Peat–Manure Composts (according to grain yield)

Variants	Top peat						Bottom peat					
	month-old composts			two-month composts			month-old composts			two-month composts		
	in g	as % of control	as % of compost	in g	as % of control	as % of compost	in g	as % of control	as % of compost	in g	as % of control	as % of compost
Control (soil)	9.6	100	—	9.6	100	—	9.6	100	—	9.6	100	—
Peat 80%+manure 20% (compost)	13.6	142	100	14.6	152	100	17.8	185	100	19.5	203	100
Compost + 2% L	18,1	188	133	20.3	211	139	19,0	200	107	22 6	235	116
Compost + 10% L	20.5	213	151	21.3	222	146	20.6	214	116	15.6	163	80
Compost + 5% RPM	21.4	223	158	16.4	171	112	20.0	208	113	18,6	194	95
Compost + 2% SP	20.6	213	151	17.5	182	120	17,3	180	97	19,8	206	101
Compost + 2% L + 5% RPM	19,3	201	142	18.3	190	125	18.5	192	104	18,6	194	95
Compost + 10% L + 5% RPM	21.6	225	159	21.1	222	144	22,0	229	123	16.6	173	85
Compost + 2% L + 2% SP	22.9	240	170	22,0	229	151	19,1	200	107	19,2	200	98
Compost + 10% L + 2% SP	20,5	213	151	24.8	258	170	20,2	210	113	21.2	221	108

butyric acid bacteria in comparison with the effect of these fertilizers added separately. In top-peat, lime and superphosphate in combination sometimes increased the multiplication of butyric acid bacteria more than lime alone.

In bottom-peat, the combined addition of 10% lime and superphosphate enhanced the multiplication of butyric acid bacteria in the second period of composting (Abyzov [1960a, b]).

One- and two-month-old composts were used in pot experiments to reveal the fertilizing value. The soil used was a poorly cultivated, light, nitrogen-poor podzol.

The experiments were set up in Kirsanov vessels of 10 kg capacity. Five hundred g of the test composts were added to the top third of the vessel and were mixed with the corresponding amount of the soil. The controls consisted of vessels with soil alone, or with soil containing added mineral fertilizers alone in amounts corresponding to the content in the composts.

Two-rowed barley of the "Viner" variety was used in the pot experiments. Similar results were obtained from duplicate experiments in two years. Table 2 shows the results for one year.

Table 2 shows that peat–manure composts composed of bottom peats had a much better effect on the crop yield than top-peat composts.

The addition of phosphate fertilizers and lime in the composting of bottom peats had much less effect than in top peats. The difference between the best variant and the control (peat–manure compost) for bottom peat did not exceed 23%, whereas the difference was nearly 100% in the case of top peat. The addition of lime increased the yield from both peats, but the increase was much greater in top peat than in bottom peat. For instance, 2% lime in top peat increased the fertilizing value of the compost by 33-39%, whereas for bottom peat the increase was 7-16%.

An increase in the dose of lime from 2 to 10% increased the yield even more, but for bottom peat it was effective only in short-term composting. Lime and rock phosphate meal in combination were ineffective in both peats and with both doses of lime. On the other hand, the addition of superphosphate and lime was very effective, especially in the composting of top peat.

The highest yields were obtained from peats containing a combination of high doses of lime and superphosphate. In the case of bottom peats the addition of superphosphate was effective only with high doses of lime and longer composting.

The obtained results also allow a comparison of the effect of lime and different forms of phosphate fertilizers in composts consisting of top or bottom peats. Lime and phosphate fertilizers, particularly rock phosphate meal, had a similar effect; hence, the addition of large doses of lime can replace phosphate fertilizers, and vice versa.

When the data of Tables 1 and 2 are compared it should be noted that although there was no strict correlation between the increase in the numbers and the increased yield there was nevertheless a definite relation between these indices.

Lime in top peat had a much greater effect on the multiplication of butyric acid bacteria than in bottom peat and, in correspondence with this, lime greatly enhanced the efficacy of top-peat composts and had much less effect on the efficacy of bottom-peat composts.

In top-peat composts containing a combination of phosphate fertilizers and lime, the increase in the numbers of bacteria was greater than in bottom-peat composts. Correspondingly, the increment of yield in the case of applying top-peat composts with phosphate fertilizers and lime in comparison with compost without fertilizers was higher than in the case of bottom-peat composts.

An increase in the numbers of bacteria was found in all top-peat composts containing mineral fertilizers and lime and, accordingly, all these variants gave an increase in yield.

This relationship could also be observed in a comparison of the individual variants. For instance, in the bottom-peat compost containing 2% lime + 2% superphosphate the numbers of bacteria did not increase, and the yield was practically the same as in the compost without fertilizer. On the other hand, in the compost containing 10% lime + 2% superphosphate the increase in the numbers of bacteria was accompanied by an increased yield.

Thus, the obtained data show that there is a definite relationship between the effect of mineral fertilizers on the development of anaerobic nitrogen-fixing bacteria in composts and the efficacy of the composts.

I express my deep thanks to Prof. E. F. Berozova for valuable comments during the conduction of this work.

SUMMARY

The effect of lime and phosphate fertilizers on the efficacy of composts is closely related to the effect of these fertilizers on the multiplication of microorganisms, particularly butyric acid bacteria, during the composting of different peats.

Lime and rock phosphate meal have a similar effect on the multiplication of the investigated bacteria and the efficacy of the composts. But the effect of these fertilizers varies with the type of peat. In top peat, lime in either dose had the best effect. In bottom peat, rock phosphate and lime in small doses were best.

Combined addition of lime and phosphate fertilizers gave good results in several cases. The addition of superphosphate to lime removed the inhibitory effect of the latter on the multiplication of butyric acid bacteria of both groups. The fertilizing value of the composts was also increased in this case.

LITERATURE CITED

1. S. S. Abyzov. 1958. Byull. nauchno-tekh. inform. po s.-kh. mikrobiol., 5: 8.
2. S. S. Abyzov. 1960a. Izvest. Akad. Nauk SSSR, Ser. Biol. 1: 154.
3. S. S. Abyzov. 1960b. Byull. nauchno-tekh. inform. po s.-kh. mikrobiol.,7: 12.
4. E. F. Berezova. 1955. Mikrobiologiya 24 (6): 723.
5. E. F. Berezova, M. I. Gusev, E. Kh. Rempe and N. I. Karpukhina. 1955. Dokl. VASKhNIL,4: 22.
6. T. D. Lysenko. 1953. Zemledelie, 5: 17.
7. A. N. Pertseva. 1950. Dokl. VASKhNIL,6, 19.
8. M. L. Stepanova, V. N. Bylinkina and N. M. Figurina, 1940. Tr. Tsentr. trof. opytn. stantsii (TsTOS) 10: 42.
9. L. A. Yung. 1950. Sov. agronomiya,3, 71.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

EFFECT OF HERBICIDES ON SOIL MICROFLORA

I. A. Geller and E. G. Khariton

All-Union Scientific Research Institute of the Sugar Beet, Kiev

Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 494-499, May-June, 1961
Original article submitted June 14, 1960

Certain concentrations of various chemical preparations used in recent years as weed control herbicides do not directly inhibit the growth and development of cultivated plants. But studies show that they do inhibit the soil microflora, and, in this way, indirectly affect plant nutrition.

Repp (1953) and Sokolov (1956) observed that 2,4-dinitrocresolate [2,4-dinitrophenoxyacetic acid](2,4-D) applied at the rate of 1-2 kg/hectare did not inhibit the soil microflora. but according to the data of Worth and McCabe (1948), this preparation inhibits the vital activity of aerobic microorganisms, shows no appreciable effect on anaerobes, and stimulates the growth of facultative anaerobes. According to Machavariani (1956), 2,4-D stimulates the development of denitrifying bacteria and inhibits nitrifying bacteria.

According to an abstract of Al'gren, Klingman, and Wolf (1953), 2,4-D, even in extremely low concentrations, inhibits tubercle formation on kidney bean roots.

In the studies of Shklyar, Voevodin, and Markova (1958), 2,4-D applied to non-sterile soil at the rate of 2.5 kg/hectare and Chloro-IPC applied at the rate of 5 kg/hectare does not inhibit the development of fungi, azotobacter, butyric acid bacteria, aerobic cellulose-decomposing bacteria, or bacteria which grow on meat-peptone agar. After sterilization of the soil, however, a marked decrease in titer of azotobacter, in comparison with the control, resulted from application of the herbicides. According to the data of Klyuchnikov and Petrova (1960), the quantity of actinomycetes in the root area of seedlings that had been treated with the herbicides 2,4-D and 2M-4C was lower than in the control. They discovered that Trichoderma predominated in the root area of the control oaklets, although encountered in smaller quantity in the oaklets treated with herbicides; members of the genus Penicillium, however, grew much more abundantly in the latter than in the control.

The grass control herbicides ammonium trichloroacetate, dichloral urea, and isopropyl N-phenylcarbamate, which belong to the root action group of herbicides, are used during cultivation of sugar beet crops. The effectiveness of these preparations depends on the method of application to the soil. Therefore, in order to use herbicides effectively during sugar beet growth, it is necessary to make allowance for the leaching of herbicides by water (Makovetskii 1960) and for the rate of herbicide deactivation, which depends on the absorbing properties of the soil and on the rate of biological processes (Dukatov, 1958; Shklyar, Voevodin, and Markova, 1958).

The sugar beet was selected for study of the effect of various concentrations of herbicides on the soil microflora because its growth and productivity are promptly decreased in response to a lack of sufficient nutritive elements in the soil, which occurs when the biological activity of the soil is reduced.*

Studies on chernozem conducted at the L'gov Experiment Station (M. M. Beregovaya) in 1958 showed that when the herbicides isopropylchlorophenylcarbamate (Chloro-IPC) and trichloroacetate were applied to the soil by cultivator at the rates of 8 and 32 kg/hectare, respectively, during soil preparation prior to seeding, the vital activity of the microorganisms was markedly inhibited (Table 1).

The herbicides referred to were applied to the soil by cultivator in early spring before seeding; their inhibiting effect on the soil microflora was quite evident as late as August and diminished only toward the end of the vegetation period, just before harvest. Herbicides do not affect the different physiological groups which comprise the soil microflora to the same extent; they markedly inhibit the vital activity of azotobacter, nitrifying bacteria, and anaerobic nitrogen fixers and reduce their number by 67 to 90%, but have less effect on the activity of cellulose-decomposing microorganisms.

By inhibiting the vital activity of soil microorganisms beneficial to plants, the herbicides in this experiment greatly reduced the sugar beet yield; this conclusion may be drawn from the data in Table 2.

From the data cited in Table 2, it is apparent that the application by cultivator of the herbicides under discussion caused a reduction in the sugar beet yield. Trichloroacetate reduced the beet yield by 60 centners/hectare and the sugar yield by 11.5 centners/hectare; Chloro-IPC reduced the beet yield by 43 centners/hectare and the sugar yield by 8.5 centners/hectare.

* Experiments at the stations were conducted in accordance with the plan of A. N. Mel'nichuk.

TABLE 1. Effect of Herbicides on the Soil Microflora (chernozem at the L'gov Experiment Station). Tested in 1958

Herbicide	Analysis date	Azotobacter, % in soil clumps	Nitrification $N-NO_3$, mg/g soil	Denitrifiers, 10^3/g soil	C. pasteurianum 10^3/g soil	Cellulose decomposition, %
Control	Aug.6	77	35,9	694.2	69,4	28.3
Chloro-IPC		24	16.3	69.9	7.0	21.6
TCA		36	29.5	29.4	7.0	24.5
Control	Oct.22	69	17.4	71.1	29.6	23.0
Chloro-IPC		68	17.1	70.8	29.5	20.6
TCA		60	17.0	70.9	7.1	21.2

TABLE 2. Effect of Herbicides on the Yield and Sugar Content of the Sugar Beet (chernozem at the L'gov Experiment Station)

Herbicide	Yield, centners/ha	Sugar, %	Sugar yield, centners/ha	Yield decrease, centners/ha
Control	293	18.7	54.8	—
Chloro-IPC	250	18.5	46.3	43
TCA	233	18.6	43.3	60

The decrease in sugar beet yield can probably be attributed to the direct effect of these preparations on the plants as well as to the inhibition of microbiological processes in the soil.

Because application of 32 kg of trichloroacetate/hectare to chernozem soil at the L'gov Experiment Station reduced the sugar beet yield and inhibited the soil microflora, smaller applications of herbicides—5 kg of dichloral urea/hectare and 8 kg of ammonium trichloroacetate/hectare—were tested at this station in 1959; these were applied by cultivator during soil preparation before seeding with sugar beet.

The results of these studies are presented in Table 3.

From the data cited in Table 3, it is apparent that small applications of herbicides (5 kg dichloralurea/hectare and 8 kg trichloroacetate/hectare) do not inhibit the soil microflora nor, judging from the data in Table 4, do they reduce the sugar beet yield.

In the Belozersk Experiment Station (T. N. Shchepak) in 1959, microbiological studies were conducted on fertile, low humus chernozem with different concentrations of herbicides. In this experiment, trichloroacetate was applied to the soil by cultivator during soil preparation before seeding with sugar beet; applications of 8, 16, and 32 kg/hectare were tested.

The results of the microbiological studies and the sugar beet yield data are presented in Table 5.

The application of 8 kg trichloroacetate/hectare to the fertile, low humus chernozem at the Belozersk Experiment Station promoted increased activity of nitrifying and ammonifying bacteria and decreased content of clostridia in the soil. The beet yield was only slightly affected. The application of 16 and 32 kg trichloroacetate/hectare caused a decrease in the activity of ammonifying bacteria and in the number of Clostridium pasteurianum cells in the soil; the activity of nitrifying bacteria and of cellulose microorganisms was decreased only when 32 kg of herbicide/hectare was applied. When the application rate was incareased to 16-32 kg/hectare, the beet yield was decreased by 18-24 centners/hectare.

One factor to be considered in the practical use of herbicides is the rate at which they become deactivated in the soil; Shklyar, Voevodin, and Markova (1958) showed that this rate depends to a great extent on the rate of microbiological processes.

According to our observations, the herbicides dichloralurea and trichloroacetate were markedly deactivated in gray podzolic soil obtained from the arable layer along the perennial weed bed, where the total quantity of bacteria was higher than in weedless fallow; this conclusion is drawn from the beet seed germination data in Table 6.

From the data cited, it is apparent that the herbicides dichloralurea and trichloroacetate decrease the germination of beet seeds in the weed beds to a smaller extent than in clean fallow; this is presumed to be due in part to the adsorption and deactivation of herbicides by organic substances and by microorganisms in the soil.

This theory has been confirmed in laboratory experiments and field tests where bacterial fertilizers (azotobacterin and phosphorobacterin) were used with the seeds and the herbicides were applied to the soil.

In a laboratory experiment (in cuvettes with four replicates), we were able to demonstrate that herbicides applied to the soil did not inhibit the seeds of winter wheat to the same extent when the seeds had been treated with azotobacterin or phosphorobacterin (figure).

When the seeds had been treated with these bacterial fertilizers, herbicide deactivation proceeded rapidly in the soil layer containing the seeds, and the germination rate of the seeds enriched with azotobacter and phosphate bacteria equaled the rate of the control.

In a field test, sodium trichloroacetate was applied

TABLE 3. Effect of Herbicides on the Soil Microflora (chernozem at the L'gov Experiment Station). Tested in 1959

Herbicide	Analysis date	Total bact., 10^6/g soil	Azotobacter, % in soil clumps	Nitrification $N-NO_3$, mg/g soil	Denitrifiers, 10^3/g soil	Cellulose decomposition, %
Control	June 8	6.43	72	28 8	80.4	42.4
DCU		7.08	80	32.8	80.2	33.9
TCA		5.90	72	28.8	81.0	33.2
Control	July 17	6.08	69	20.9	71.6	26.1
DCU		5.99	72	21.1	70.5	24.8
TCA		6.17	68	20.9	71.2	25.9
Control	Aug.16	4.32	52	11.4	28.4	22.0
DCU		4.61	50	6.5	28.8	23.2
TCA		4.44	61	6.1	28.5	21.9

TABLE 4. Effect of Herbicides on the Sugar Beet Yield (chernozem at the L'gov Experiment Station). Tested in 1959

Herbicide	Root yield, centners/hectare	Sugar, %	Sugar yield, centners/hectare
Control	343	18.4	63.1
Dichloralurea	340	19.2	65.3
Trichloroacetate	336	19.6	65.9

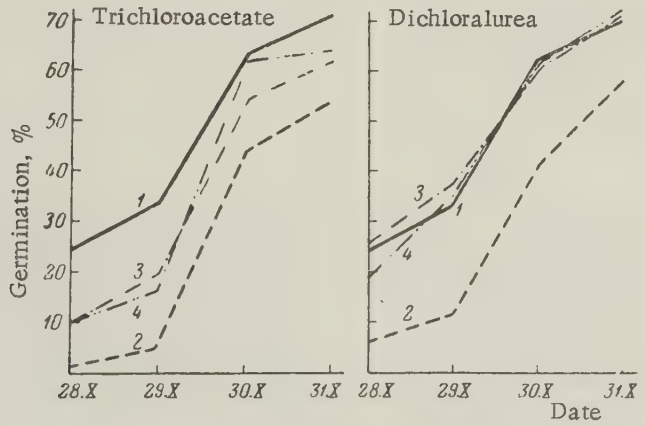
to gray podzolic soil at the rate of 30 kg/hectare during soil preparation prior to seeding, and the bacterial fertilizers azotobacterin and phosphorobacterin were applied with the seeds.

The results of this experiment indicated that sodium trichloroacetate applied at the rate of 30 kg/hectare inhibited the soil microflora and reduced the beet yield.

Applications of the above bacterial fertilizers with the seeds and of the herbicides by cultivator promoted increased activity of the soil microflora; the sugar beet yield in this case was equal to that of the control (Table 7).

In summary, it can be stated that to develop effective methods of herbicide application, the direct effect of the herbicides on the soil microflora as well as on the crops and weeds, and the effect of the soil microflora on the herbicide deactivation rate, must be taken into consideration.

Studies have shown that small applications of herbicides (5-8 kg of dichloralurea or trichloroacetate/hectare) do not greatly inhibit the vital activity of the soil microflora or weed growth. In experiments on terrace chernozem at the Pervomaisk Experiment Station, application of trichloroacetate by cultivator at the rate of 6 kg/hectare reduced the number of weeds until the beet plants were thinned out (from 33 to 15 sq. meter); an application of 24 kg/hectare was required to attain



Effect of bacterial fertilizers on the germination of winter wheat seeds after application of herbicides. 1) Control; 2) herbicide; 3) herbicide + azotobacter; 4) herbicide + phosphate bacteria.

TABLE 5. Effect of Various Doses of Herbicides on the Soil Microflora and the Sugar Beet Yield (fertile, low-humus chernozem at the Belozersk Experiment Station). Tested in 1959

Herbicide	Ammonification $N-NH_3$, mg/g soil	Nitrification $N-NO_3$, mg/g soil	C. pasteurianum, 10^3/g soil	Cellulose decomposition, %	Root yield, centners/ha	Sugar, %	Sugar yield, centners/ha
Control	5.27	28.73	13.43	17.33	306	19.7	60.28
8 kg/ha	5.97	31.50	4.27	17.17	311	19.4	60.33
TCA 16 kg/ha	4.73	31.83	2.27	17.50	288	18.4	52.99
32 kg/ha	4.33	19.80	—	15.50	282	19.6	55.27

TABLE 6. Effect of Dichloralurea and Trichloroacetate on Beet Seed Germination in a Weed Bed and in Weedless Soil

Herbicide	Quantity of intergrown lumps, %	
	Arable soil in weed bed	Arable, weedless soil
Control	87	77
Dichoralurea	82	54
Trichloroacetate	74	39

TABLE 7. Effect of Herbicides on the Soil Microflora and on the Sugar Beet Yield when Bacterial Fertilizers are Applied. (Gray podzolic soil. Tested in 1959)

Variant	Total bact., 10^6/g soil	Nitrification $N-NO_3$, mg/g soil	Cellulose decomposition, %	Yield, centners/ha	Sugar, %	Sugar yield, centners/ha
Control	15.30	3.15	31.16	200	20.7	41.40
Trichloroacetate, 30 kg/ha	8.75	2.20	31.78	179	19.6	35.08
Same + azotobacterin with seed	21.32	4.00	46.80	202	20.9	42.22
Same + phosphorobacterin with seed	13.38	5.10	32.99	200	21.0	42.00

almost complete eradication of the weeds.

The vital activity of some groups of microorganisms useful to plants is inhibited by larger applications of herbicides; this is one factor in decreased sugar beet productivity.

In selecting the optimal application rate for herbicides, it is necessary to make allowance for the deactivation of some herbicides (dichloralurea) which is the result of biological reactions and of absorption by the soil. Therefore, alteration of the biological activity of the soil, and in particular by bacterial fertilizer application (azobacterin and phosphorobacterin) may result in the partial deactivation of the herbicides in the layer of soil in direct contact with the seeds and in a consequent increase in germination; in the field test on gray podzolic soil, the inhibiting effect produced by large applications of herbicides on the productive capacity of the sugar beet was completely eliminated when these bacterial fertilizers were applied.

SUMMARY

1. In the gray podzolic soils, fertile low humus soils, and chernozems, the herbicides ammonium trichloroacetate, dichloralurea, and isopropylchlorophenyl carbamate applied at rates exceeding 5-8 kg/hectare reduced the

activity of ammonifying and nitrifying bacteria and the content of azobacter and Clostridium pasteurianum in the soil.

2. The herbicides applied to the soil were gradually deactivated. The vital activity of soil microorganisms was one of several factors causing deactivation. The increased biological activity in the layer of soil in direct contact with the seeds which had been treated with the bacterial fertilizers azobacterin and phosphorobacterin accelerated herbicide deactivation, increased the seed germination rate, and raised the sugar beet yield to the level of the controls.

LITERATURE CITED

1. D. T. Algren, G. Klingman and D. Wolf, 1953, Weed Control (Izd. Inost. Lit., Moscow, 1958) p. 163.
2. N. E. Dukatov, 1958, Chemical Method of Weed Control in Forestry [in Russian] p. 46.
3. L. Yu. Klyuchnikov and A. N. Petrova, 1960, Mikrobiologiya 29 (2): 238.
4. K. O. Makovetskii, 1960, Dopovidi Ukr. Akad. s.-g. nauk., No. 2, 25.
5. Machavariani, 1954, Tr. In-ta Polev. An GruzSSR 8, 99; R. Zh. Biologiya, No. 6, 22513 (1956).
6. G. Repp, 1956, R. Zh. Biologiya, No. 6, 22512.
7. N. S. Sokolov, 1956, Agriculture [in Russian] No. 7, 98.
8. M. Z. Shklyar, A. V. Voevodin and V. G. Markova, 1958, Byull. n-tekh. inform. po s.-kh. mikrobiol., No. 4, 14.
9. W. A. Worth and A. M. McCabe, 1948, Science 108, 16.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

MICROBIOLOGICAL STUDIES OF CARPATHIAN SULFUR DEPOSITS

V. Occurrence of Sulfate-Reducing Bacteria in Sedimentary Rocks Composing Sulfur Deposits

M. V. Ivanov

Institute of Microbiology, Academy of Sciences, USSR
Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 500-502, May-June, 1961
Original article submitted March 13, 1960

In investigations of the role of microorganisms in the cycle of substances in the soil considerable attention is being given to a study of the effect of adsorption processes on the activity of soil microorganisms. On the other hand, research workers engaged in investigations of the activity of microorganisms in underground waters usually analyze only water samples, and give much less attention to the investigation of the microflora of the containing rocks.

Yet it is quite clear that a great part in the formation and alteration of the chemical composition of underground waters is played by the microflora of the solid phase of the underground water reservoir.

Of particular importance is an investigation of the microflora adsorbed by the rock in the case of studies of the activity of sulfate-reducing bacteria, which, as Rubenchik, Roizin, and Bilyanskii [1934] showed, are more active biochemical agents in the adsorbed state.

In view of this information, in our investigation of the role of microorganisms in underground waters of the Carpathian sulfur deposits we devoted considerable attention to a study of the occurance of sulfate-reducing bacteria not only in the waters, but also in the rocks composing these deposits.

The results of these investigations are given in this paper.

The inocula consisted of the material of cores obtained in the drilling of test and observation wells. We examined all the rocks comprising the normal geographical profile typical of the sulfur deposits of the Carpathian region—Quaternary deposits, the Upper Tortonian and Sarmatian clay-marl layer, the Upper Tortonian limestones and gypsums, and the Lower Tortonian Lithothamnium limestones and sandstones (Kudrin [1957]). We paid most attention to a study of the Upper Tortonian sulfur-bearing limestones and their underlying gypsum—anhydrites.

The cores, taken immediately after the wells were drilled, were broken up in the laboratory and material from the center was used to inoculate agar Tauson medium as modified by Shturm. The samples were incubated at 28° for two months. The growth of sulfate-reducing bacteria was ascertained from the blackening of the medium due to the production of hydrogen sulfide from the sulfates and the precipitation of FeS.

All the data obtained in the investigation of 56 rock samples from nine wells in different fields are given in Fig. 1, and the summarized data for the various deposits are given in Fig. 2.

The most noteworthy feature of the obtained data is the wide occurance of sulfate-reducing bacteria in sulfur-bearing limestones. These bacteria were found in every sample, except the two (29 and 34) taken from the upper part of the sulfur-bearing horizon. In this connection it is interesting to note that in other wells also (Yazov 53, Yazov 52, Rozdol 1p) the upper parts of the limestone horizon did not contain sulfate-reducing bacteria. The explanation of this should probably be sought in the mechanical properties of the limestone, the most compact varieties of which are found in the upper part of the horizon. Such structures do not favor the circulation of underground waters and this is evidently why compact varieties of limestones contain fewer bacteria. In those wells where the limestones were cavernous in the upper part too (well 37G) or karstic and bedded in clay (Lyuben'-Velikii, well 2069), sulfate-reducing bacteria were found also in the upper parts of the limestone horizon (samples 18 and 41).

The dependence of the distribution of sulfate-reducing bacteria on the porosity and fracturing of the rock is even more clearly seen in their frequency of occurrence in gypsum samples.

Figure 2 shows that these bacteria were isolated from almost all the samples of sulfur-containing gyspum, whereas none of the sulfur-free gypsum samples gave any growth (samples 12, 13, 39—Fig. 1). Yet we know that the presence of sulfur in the gypsum of the Carpathian fields is of a secondary nature and occurs only in porous and fissured regions of the gypsum—anhydrite

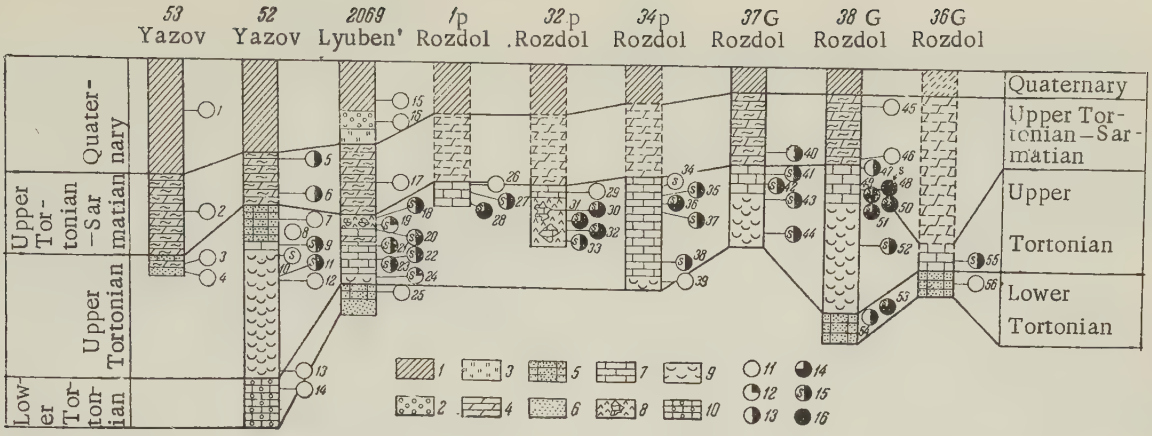


Fig. 1. Occurrence of sulfate-reducing bacteria in sedimentary rocks of Carpathian sulfur deposits. 1) Quaternary deposits (loams and sandy loams); 2) ferruginous clays; 3) fossil peat; 4) Sarmatian and Upper Tortonian clay-marl layer; 5) sandstone; 6) sand; 7) Upper Tortonian, principal sulfur-bearing horizon; 8) disintegrated sulfur-bearing limestone in clay; 9) gypsum–anhydrite layer; 10) Lithothamnium limestone; 11) rock samples which gave no growth when inoculated onto medium for sulfate-reducing bacteria; 12) rock samples which gave growth with undiluted inoculum; 13) rock samples which gave growth in first dilution; 14) rock samples which gave growth with inoculum in second dilution; 15) letter S denotes samples of sulfur-bearing rocks; 16) samples which gave growth with inoculum in third dilution.

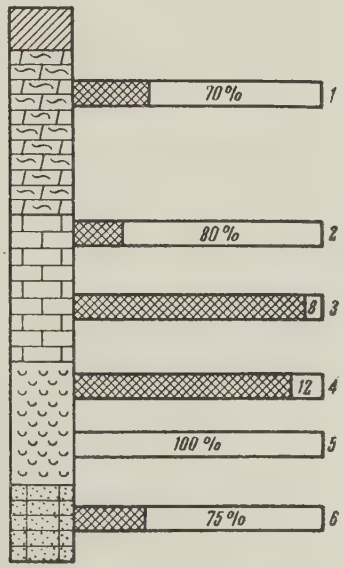


Fig. 2. Summarized data on occurence of sulfate-reducing bacteria in rock samples from Carpathian sulfur deposits. Cross hatched columns – percentage of samples in which bacteria were found. Symbols for rocks as in Fig. 1. 1) Quaternary rocks and Upper Tortonian and Sarmatian clayey layer; 2) sulfur-free rocks of limestone horizon; 3) sulfur-bearing Upper Tortonian limestones; 4) samples of sulfur-containing gypsums; 5) samples of compact, sulfur-free gypsums; 6) Lower Tortonian Lithothamnium limestones and sandstones.

layer, i. e., again in zones of migration of underground waters.

Thus, the data on the occurence of sulfate-reducing bacteria in sedimentary rocks of the Carpathian sulfur deposits show that these bacteria are most widely distributed in porous and fissured Upper Tortonian rocks through which hydrogen sulfide waters circulate. In such rocks bacteria were found in 90% of the investigated samples (Fig. 2, 3 and 4). In compact, sulfur-free regions of the Upper Tortonian sulfur-bearing horizon (Fig. 2, 2 and 5), and also in the clay-marl layer overlying the limestone and in the Lower Tortonian rocks underlying the deposit, sulfate-reducing bacteria occurred much more rarely (Fig. 2, 1 and 6).

The number of sulfate-reducing bacteria in most of the investigated samples exceeded 10-100 cells per g of rock (Fig. 1). Comparing these data with the results of a study of the occurrence of sulfate-reducing bacteria in underground waters of sulfur deposits of this region (Ivanov and Kostruba [1961]; Ivanov and Ryzhova [1961]), we see that the number of sulfate-reducing bacteria in the rocks in most cases is one or two orders higher than their number in waters.

SUMMARY

1. Sulfate-reducing bacteria are widely distributed in porous and fissured varieties of the Upper Tortonian rocks composing the Carpathian sulfur deposits.

2. In rocks overlying and underlying the sulfur deposits, and also in the less porous samples of sulfur-containing rocks, sulfate-reducing bacteria are less frequently found and less abundant.

LITERATURE CITED

1. M. V. Ivanov and M. F. Kostruba, 1961, Mikrobiologiya 30, 1.
2. M. V. Ivanov and V. N. Ryzhova, 1961, Mikrobiologiya 30, 2.
3. L. N. Kudrin, 1957, Geol. Zh. AN Ukr. SSSR 17, 4.
4. L. I. Rubenchik, M. B. Roizin and F. M. Bilyanskii, 1934, Mikrobiologiya 3, 3.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-tc-cover English translations appears at the back of this issue.

OCCURRENCE OF Thiobacillus thioparus IN HYDROGEN SULFIDE UNDERGROUND WATERS

G. A. Sokolova

Institute of Microbiology, Academy of Sciences, USSR
Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 503-510, May-June, 1961
Original article submitted May 6, 1960

One of the principal organisms involved in the sulfur cylce in nature is Thiobacillus thioparus.

As Ivanov's investigations [1957] showed, this organism plays an important role in the formation of epigenetic sulfur deposits.

Several authors (Ginzberg-Karagicheva [1926], Kuznetsova [1961], and others) have recorded the presence of this organism in oil-seam waters, where dissolved oxygen is practically absent.

Our investigations on the physiological characters of T. thioparus (Kuznetsov and Sokolova [1960]) showed that this organism is not a strict aerobe, as was earlier believed. The oxidation of hyposulfite with the production of molecular sulfur in a culture of these bacteria is most rapid when a little oxygen is available, when the rH_2 of the medium is 10-16.

But there is still no clear explanation of the presence of this organism in anaerobic conditions in oil fields. Hence, the aim of the present work was to investigate the main factors affecting the distribution of T. thioparus in natural conditions.

It might be assumed that the occurence of this organism must be due to the presence of hydrogen sulfide as an oxidizable substrate and energy source and to the presence of oxygen as an oxidant.

It would appear that where free hydrogen sulfide or sulfides are present in water, as is the case in oil fields, the presence of free oxygen is out of the question. In this case we might infer that the latter is brought into the deep layers of water either by surface waters from the supply region of water-bearing strata, or by surface waters pumped into the field when methods of secondary recovery of oil are used. In these cases analytically free oxygen may not be found in the seam waters, since it will be used up in the oxidation of hydrogen sulfide in the region where the deep and surface waters meet.

In connection with the above we studied the distribution of T. thioparus in the Yazov and Rozdol sulfur fields and in several oil fields with different types of water exchange in Kuibyshev and Orenburg Regions.

METHODS

In the sulfur fields water samples were taken from operating drainage wells or true artesian observation wells. In the oil fields water samples were taken mainly from the sampling cocks of producing wells and in a few cases from subartesian or idle wells by a bailer.

The thiobacteria were counted by the serial dilution method in Beijerinck's medium. In the analysis of the numbers of bacteria in highly-mineralized oil seam waters, the nutrient medium was prepared with water diluted with an equal volume of the seam water from which the inoculum was taken. The inoculated samples were kept for about two months before we finally decided that there was no growth of bacteria.

Hydrogen sulfide was determined by iodometric titration directly at the sampling site; the pH was determined colorimetrically or with a glass electrode on the P-6 potentiometer. The oxidation–reduction potential was measured with a smooth platinum electrode. We also used the data of Kuznetsova [1960] and several chemical laboratories on the oxidation–reduction potential and salts composition of seam waters.

EXPERIMENTAL

According to existing theories, the oil from the mother rocks migrates through fissures and, on reaching the water-bearing horizons, collects in oil traps (Fig. 1). In open deposits the surface waters from the supply region flow into the unloading region through the water-bearing horizon, and if the supply zones are not far from the field they come into contact with the oil deposit (Groups I and II in table). In other cases the activity of sulfate-reducing bacteria gives rise to calcite, which is deposited and completely seals the underlying rocks, thus separating the oil deposits from the water-bearing horizon. Such deposits are called sealed deposits (Groups III and IV).

As the oil reserves are used up in the exploitation of the fields the pressure in the seam falls and it must be increased artificially by injection of water into the oil seam.

In sealed deposits the water is pumped directly into the oil pool and, for maintenance of the gas pressure, the first producing wells are bored into the boundary zone (Fig. 1). In open deposits the water is pumped into the region outside the pool.

The results of the investigation on the occurrence of T. thioparus in these groups of deposits are given in the table.

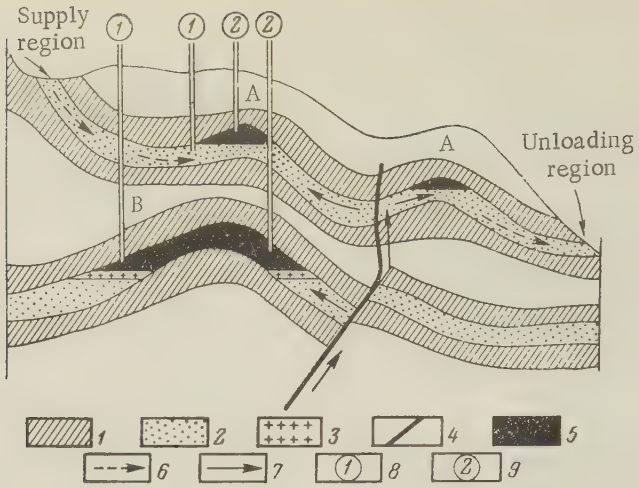


Fig. 1. Diagram of open (A) and sealed (B) oil deposits. 1) Impermeable rocks; 2) permeable rocks; 3) deposits of secondary calcite; 4) fissures through which oil migrates; 5) oil pool; 6) path of water; 7) path of oil; 8) water-drive well; 9) producing well.

Occurence of Thiobacillus thioparus in Seam Waters of Oil Fields

Type of deposit	Field	Stratigraphic horizon	Mean density of seam water	Hydrogen sulfide content, mg/liter	pH	Number of samples	Number of samples containing T. thioparus
I. Open deposits with injection of fresh water	Kalinovka	P_2	1.090	224-1261	7.4-7.6	34	16
	Novostepanovka	P_2	1.008	700		2	2
	Zol'nyi ovrag	$C(B_2)$	1.170	present		2	1*
	Yablonovyi ovrag	$C(B_2)$	1.169	12-168	6 and < 6	3	0
	Syzran'	$C(B_1$ and $B_2)$	1.090	204-332	6.2-6.9	6	2
	Novostepanovka	C_1		699	7.6	1	0
	Mukhanovo	C_1	1.186			1	1
	Yablonovyi ovrag	D_1-D_3	1.175	0	6	6	0
II. Open deposits with no injection of fresh water	Radaevka	$C(B_2)$	1.159	19-100	6.0-6.4	3	3*
	Pokrovskoe	$C(B_2)$	1.134	325-348	6.0-6.2	2	0
	Mukhanovo	D_1	1.175	0	6	1	0
	Sadki	D_3	1.173	0	6	1	0
III. Sealed deposits with injection of fresh water	Pokrovskoe	$C(A_4)$	1.028	43-74	7.0-7.5	2	2
	Yablonya	P_1	1.115	25-100	6.2-6.5	7	5
IV. Sealed deposits with no injection of fresh water	Kokhany	P_1	1.154	0	–	2	2*
	Gorodetskaya pl.	P_1	1.159	present	6.8	2	1**
	Yablonya	P_1	1.184	present		4	0
	Krasnoyarka	C_1	1.196			2	0
	Zol'nyi ovrag	D_1	1.173	0	6	7	0

*Samples taken by bailer.

**Leakage of water through casing from outside, sample taken by bailer.

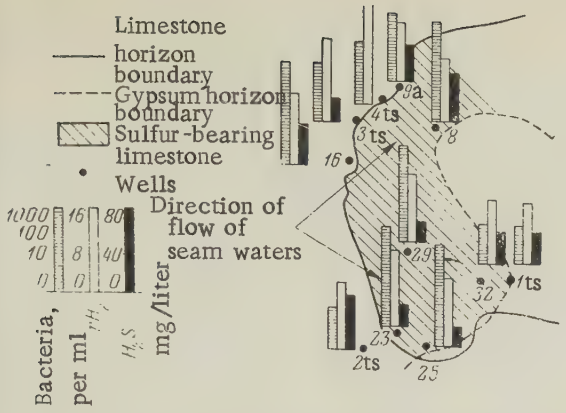


Fig. 2. Occurence of Thiobacillus thioparus in relation to hydrogen sulfide content and rH_2 in seam waters of Rozdol sulfur field.

As the table shows, thiobacteria were found, despite the varied composition of the seam waters, in almost all the wells where injection was being conducted. The Kalinovka field was studied more thoroughly. This is an old oil field, heavily flooded by the injection of water. In the northwestern part the structure of the oil-bearing seam has been destroyed and there is an inflow of fresh sulfate waters from the Permian deposits. Hence, the chemical composition of the water varies from a sodium-sulfate type in the western part to a magnesium-chloride type in the east. Mineralization increases in the same direction.

The most highly mineralized waters are found in the northern part of the field. The hydrogen sulfide content is high and varies from 224 to 1260 mg/liter. The oxidation–reduction potential varies from 4.5 to 7.6. Despite such a diversity of conditions and the low oxidation–reduction potential, thiobacteria were found in most of the samples from the whole area, although in small numbers (1-10 cells per ml), as in the other samples from oil seam waters.

No thiobacteria were found in the seam waters of group II deposits, except Radaevka, where the samples were taken by a bailer from subartesian and abandoned wells and contamination of the samples was possible. Presumably because the water supply regions of oil deposits of this type are situated a considerable distance away, the water entering the oil pool is devoid of oxygen and the enviromental conditions do not favor the development of T. thioparus.

Thiobacteria were also found only in sealed deposits where water injection was being carried out and the entrance of oxygen into the seam was possible (group III). In deposits of the fourth group T. thioparus was found only in the sample from Gorodetskaya Ploshchad', well No. 300, where there was an inflow of water from the space around the casing.

Interesting results were obtained in Pokrovskoe field. In this field fresh artesian water, which does not contain these bacteria, is being pumped into two exploited pools–A_4 and B_2. Pool A_4 is sealed. The water is highly freshened, there is little hydrogen sulfide, and thiobacteria are very active–up to 100 cells per ml. Pool B_2 is of the open type. Despite the fact that flooding has been in operation for a long time, the front of the injected water is advancing very slowly from the injection site owing to the lack of fissures in the rocks. Hence, the mineralization remains high, i. e., there is practically no dilution of the seam water by the fresh water, and thiobacteria were absent here.

In the Yablonya sealed deposit, where water is being injected over the whole area, the seam waters are appreciably freshened, except in a few regions where the mineralization remains high. T. thioparus was not found in samples from these regions, but was present in the other samples.

As the table shows, T. thioparus does not develop in Devonian waters owing to the absence of hydrogen sulfide in them.

We should note the presence of these bacteria in the highly mineralized Carboniferous waters of Mukhanovo, Zol'nyi ovrag, etc. The pure cultures of bacteria isolated from seam waters of Kalinovka were obligate halophiles, capable of tolerating considerable concentrations of salt (up to 150 g NaCl per liter, although the mineral content of the seam water was 76 g/liter). This indicates that they are not brought into the seam from outside with the waters flowing into it, but that they develop in the seam itself and have become adapted to a high concentration of salts.

Thus, thiobacteria were found only in those deposits where fresh water supplying the oxygen necessary for their activity was being injected.

The Carpathian sulfur fields are interesting in that the supply zones are not remote from the deposits and the waters still contain free oxygen on entry into the seam of the sulfur deposit. Hence, the change in the seam waters as they advance through the deposit can be fully studied here. The water here is weakly mineralized, the water exchange is more rapid than in the oil fields, and the total numbers of thiobacteria are much higher.

A full hydrogeological characterization of the Rozdol and Yazov fields was given in Ivanov's paper [1960].

Rozdol field is distinguished by the high rate of water exchange owing to the industrial extraction of water. From the start of operation in 1951 the static water supply has been reduced by more than four-fifths.

The formation of the waters of the limestone horizon proceeds as they advance in the two directions indicated in Fig. 2. Mineralization increases in the same direction. The hydrogen sulfide content increases and the oxidation – reduction potential falls from rH_2 18 to 10, i. e., the conditions become more anaerobic. It

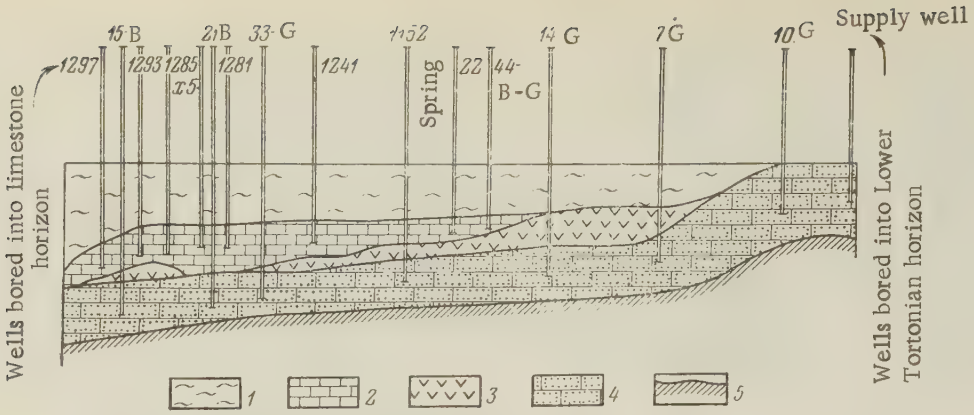


Fig. 3. Stratigraphic section of Yazov sulfur field. 1) Tortonian-Sarmatian clay layer; 2) sulfur-bearing Ratinian limestone; 3) Upper Tortonian gypsum–anhydrite; 4) Lower Tortonian limestone and sandstone; 5) top of Upper Cretaceous layers.

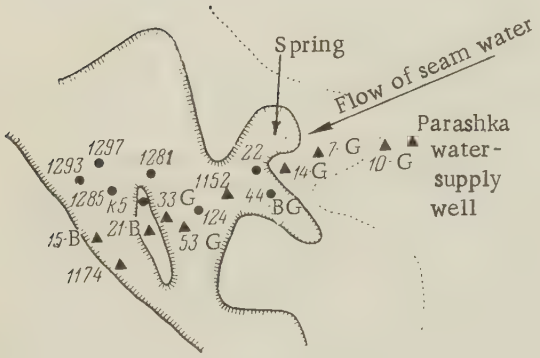


Fig. 4. Diagram of Yazov sulfur field.

was found that thiobacteria responded very distinctly to change in the seam water, and the number of bacteria diminished from 100 thous cells per ml of water in wells 16, 29a, and 23 at the entrance to the field to 100 cells per ml in wells 32 and 1ts at the outlet. Apparently the most favorable conditions for their development are created at the boundary between the oxygen-containing waters and the H_2S-containing waters, where the oxidation–reduction potential is low.

As observations showed, well 2ts is located in a region where water exchange is hindered, since here we noted the lowest oxidation–reduction potential, the highest hydrogen sulfide content and the greatest mineralization in comparison with nearby wells. In view of these factors the number of thiobacteria here was also relatively low.

It should be noted that, despite the fairly low rH_2 values (12-14) in the seam waters of Rozdol field, T. thioparus was found everywhere in considerable numbers, at least 100 cells per ml of water. This can be explained, as already indicated, by the rapid flow of water through the limestone horizon.

The Yazov field is not yet being exploited. No water is being pumped out. The area is covered with a network of observation wells bored into the sulfur-bearing limestone and Lower Tortonian horizons (Figs. 3 and 4). The zone of influx of surface waters is situated in the region of the Parashka water supply well, where the overlying impermeable clay strata taper off and the Tortonian deposits emerge from directly under the Quaternary deposits. The water flows in the direction of a transect between wells 14G and 33G, as Fig. 3 shows. The limestone horizon is fed by Lower Tortonian waters, which penetrate into the limestone horizon through cracks, "windows," etc., in the underlying gypsum. The water in the gypsum flows in the same direction.

It is of interest to trace the changes in the oxidation–reduction potential as the waters pass through these two horizons. In wells 7G, 14G, 10G the water contains a lot of oxygen and has a high potential ($rH_2 = 26$), which gradually falls to rH_2 9-10 at wells 33G and 21B, where the hydrogen sulfide content reaches more than 100 mg/liter. Thiobacteria were found in the waters entering the field (up to 100 cell per ml), but in waters which had passed through the whole field (well 21B) their numbers were reduced (Fig. 5).

The water of the limestone horizon (Fig. 6) flows in the same direction. Since waters devoid of oxygen and with a lower oxidation–reduction potential enter the layer of sulfur-bearing limestone from below, hydrogen sulfide is found in well 22, situated at the entry into the field, while in well 1297 the H_2S content reaches 120 mg/liter and the rH_2 is reduced to 8, i. e., conditions here are even more anaerobic than in the Lower Tortonian waters and there are no thiobacteria in wells 1293 and 1297.

Ivanov [1961] investigated another two wells–38G and 24G– sunk into the Lower Tortonian deposits in the region of well 15B. The water from them contained up to 515 mg H_2S/liter, had a very low oxidation–reduction potential, and was distinguished by a high chlorine content, due presumably to an influx of chlorine waters from the Lower Cretaceous deposits, which do not contain hydrogen sulfide and, possibly, contain oxygen. Thiobac -

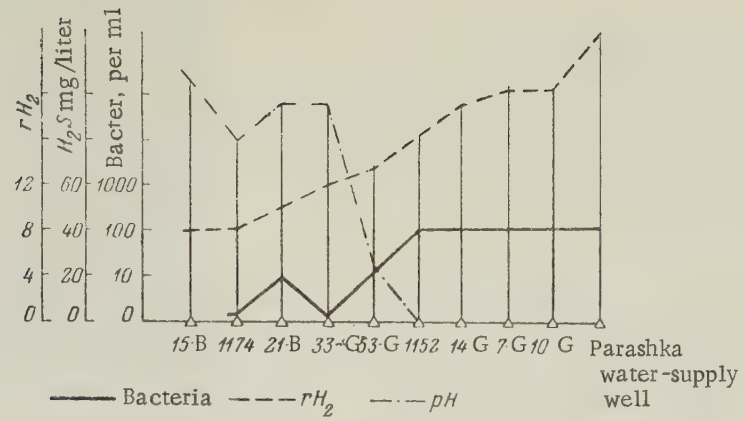


Fig. 5. Occurence of Thiobacillus thioparus in relation to hydrogen sulfide content and rH_2 of seam waters in Lower Tortonian horizon of Yazov sulfur field.

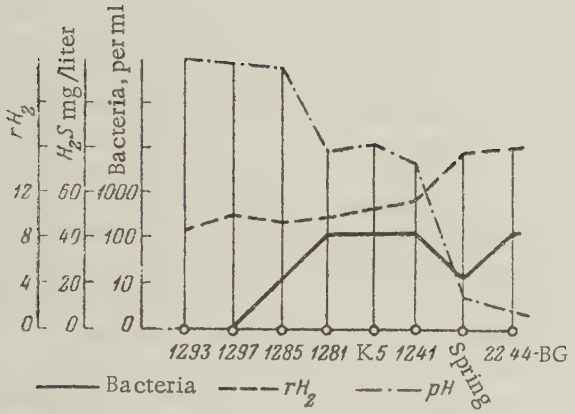


Fig. 6. Occurence of Thiobacillus thioparus in relation to rH_2 and hydrogen sulfide content in seam waters of limestone horizon of Yazov field.

teria respond with intense growth to the influx of these waters.

Thus, in the seam waters both of oil fields and sulfur fields we find a close relationship between the occurrence of T. thioparus and the penetration of oxygen into the zone of H_2S-containing waters, and these bacteria are found even where the oxidation–reduction potential is very low and the hydrogen sulfide content is high.

In view of this they can probably serve as an indicator of the penetration of fresh oxygen-containing waters into deep hydrogen-sulfide waters.

SUMMARY

A study of the occurence of Thiobacillus thioparus in hydrogen sulfide fields has shown that they occur only where there is an influx of oxygen-containing water. Hence, their presence in deep hydrogen sulfide waters can apparently serve as an indicator of the influx of oxygen-containing water.

Thiobacteria isolated from highly mineralized oil-field seam waters are obligate halophiles. This indicates that they are not brought into the seam from outside by the waters flowing into it, but that they develop in it and over a long period have adapted to a high concentration of salts.

The presence of Thiobacillus thioparus in Permian and Carboniferous deposits is not affected by the chemical composition of the water. They can develop at low oxidation – reduction potential and high hydrogen sulfide content. They are absent from Devonian waters owing to the absence of hydrogen sulfide.

The greater development of Thiobacillus thioparus in waters of the Rozdol sulfur field as compared with the Yazov field is presumably due to the more rapid water exchange and, consequently, the greater availability of oxygen.

LITERATURE CITED

1. T. L. Ginzburg-Karagicheva, 1926, Azerb. neft. khoz., 6-7, 30.
2. M. V. Ivanov, 1957, Mikrobiologiya 26, 5.
3. M. V. Ivanov, 1960, Mikrobiologiya 29, 2.
3. Z. I. Kuznetsova, 1961, Tr. INMI, 9.
4. S. I. Kuznetsov and G. A. Sokolova, 1960, Mikrobiologiya 29, 3.
5. M. V. Ivanov, 1961, Mikrobiologiya 30, 3.

ROLE OF MICROORGANISMS IN IRON CORROSION IN WATER

V. V. Kuznetsov and L. V. Verzhbitskaya

Natural Science Institute, A. M. Gor'kii Perm State University
Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 511-514, May-June, 1961
Original article submitted May 6, 1960

During an investigation of the causes of intense pitting corrosion of aggregates and metal structures on the Kama Hydroelectric Station it became necessary to carry out microbiological studies to assess the role of biogenic factors in the development of corrosion. This was dictated by the fact that the foci of corrosion outwardly resembled the excresent concretions formed in media containing iron bacteria. On the surface of the weir locks, the locks of the turbine chambers, in the spiral casings, and on other objects we found many convex friable formations (excrescences) of layered structure and of varying size(figure). The excrescences were easily detached from the surface. On the locks the excrescences were larger (the base diameter was 1.5 to 7 cm): there were 10 to 15 excrescences per dm^2. Under all the excrescences there were pits 1 to 3 mm deep in the metal. The greatest concentration of foci was observed in places where the metal had been cut and welded, in the region of seams and in grooves. Such destruction of the metal had occurred after the metal structures had been in use for 2-3 years.

Before operation commenced the structures were given one coat of bituminous paint. The metal structures were made from st. 3 steel.

The microbiologist K. F. Filippova made a microbiological study of the Kama water. The samples were taken from depths 1, 5, and 10 m from the dividing piers of the dam and from the surface of the weir sluice-gate. All the water samples, particularly those from the surface of the metal sluice-gate, contained large numbers of iron bacteria—Leptothrix (two species), Crenothrix and Spirophylum ferrugineum. Iron bacteria were also found in tap water, but in smaller numbers. They developed abundantly in all the specially prepared media.

The investigation would be far from complete if we ignored other possible causes of the corrosion of metal structures. In this connection we carefully studied the fluctuations of the mineral content of Kama water in winter and summer, the oxygen and temperature conditions, and the organic matter content. We conducted laboratory and field investigations on the corrosion of samples of st. 3 steel. We studied the features and rate of corrosion of painted and unpainted specimens of steel in distilled and tap water, and in 3% sodium chloride solution. Finally, we conducted a series of tests with zinc protectors.

During the investigations we found that the water of the middle Kama was distinguished by a relatively high mineral content in comparison with other large fresh waters of the USSR. For instance, the total mineral content of Kama water, according to the data of special analyses conducted in March, 1959, reached 460-470 mg/liter; in summer the mineral content fell to 180-220 mg/liter. The water contained a large amount of chlorides and sulfates. In March, 1959, the Cl' concentration in different water samples varied from 90 to 198 mg/liter, and that of SO_4'' from 42 to 94 mg/liter. In the Dnieper near the Lenin Hydroelectric Station dam, according to 1952 data, the water contained 4 to 16 mg of Cl' per liter, and 3 to 18 mg of SO_4'' per liter. In the region of the Kama Hydroelectric Station the chloride content was higher near the right bank of the Kama, and the sulfate content was higher near the left bank. During the ice cover the composition of the water varied with the depth. Chlorides predominated in the surface layers, and sulfates predominated in the deep layers. The high mineral content of the water and the increased Cl' content is due to the pollution of the river by effluents from industrial plants. The high sulfate content of the water is due to the transport of salts into the Kama by its left tributary, the R. Chusovaya, which drains the gypsum-bearing strata of the Kungurian series. The R. Chusovaya enters the Kama 4 km above the dam.

The oxygen content of the water in summer varied from 70 to 80% of saturation, and in winter from 20 to 55% of saturation. A very important fact was the variation of oxygen content with depth just next to the dam.

The organic matter content of the water was 52-72 mg/liter and remained almost constant throughout the year.

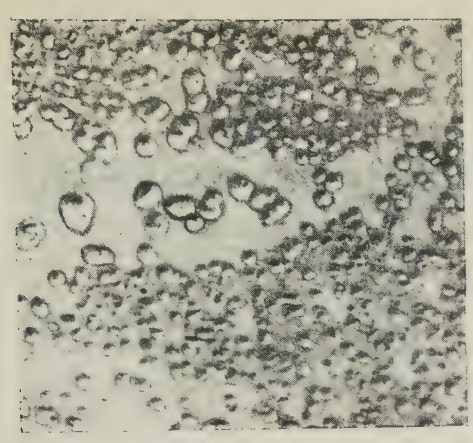
These data show that the Kama water is aggressive and is a favorable medium for the development of electrochemical corrosion.

What role do biogenic and electrochemical factors play in corrosion?

In the literature there are two quite different views on the question of biogenic corrosion. According to the view of S. N. Winogradsky and N. G. Kholodnyi, iron bacteria develop by chemosynthesis in accordance with the following reaction:

$$2FeCO_3 + 3H_2O + \tfrac{1}{2}O_2 = 2Fe(OH)_3 + 2CO_2 + 29000 \text{ cal}$$

Iron bacteria oxidize carbonates of ferrous oxide. The ferric hydroxide produced accumulates in the form of a deposit on the corroding surface. Iron bacteria actively develop in media containing iron but not containing or-



ganic matter. If the latter is present the iron bacteria die.

The other point of view is developed in Kalinenko's papers [1946, 1949, 1956]. This author notes that iron bacteria do not die in the presence of organic matter but, on the contrary, actively develop in such media. Iron bacteria develop equally well in media containing iron and media devoid of iron. Thus, iron does not play any role in their physiology. Iron bacteria can only adsorb ready forms of iron—organic, colloidally dispersed, etc.—and also compounds of calcium, magnesium, silicon, and manganese. As a result of the adsorption of different forms of iron and other compounds and the death of the iron bacteria cells, a deposit consisting of flat and convex concentrations forms on the surface. From many years' investigations Kalinenko concludes that iron bacteria are not the primary cause of metal destruction during corrosion. The corrosion of metal articles in media containing iron bacteria is a result of electrochemical processes which alter the state of the iron whether microorganisms are present or not.

From the analysis of our data on the corrosive medium in the region of the hydroelectric station we can conclude that the intensive pitting corrosion of aggregates and metal structures of the Kama Hydroelectric Station is a result of vigorous electrochemical processes. On metal surfaces in water with a high mineral content and particular oxygen conditions a favorable environment is created for the occurrence of long-lived local potential differences. The simultaneous presence of passivator (oxygen) in insufficient amount and activator (Cl') also creates conditions for the initiation of focal corrosion penetrating deeply into the metal. The further development of focal corrosion is due to the formation of insoluble corrosion products. Under the layer of these breakdown products, by the mechanism of differential aeration (access of oxygen to the metal surface under the corrosion products is limited and such parts become anodes), anodic solution of the steel takes place. The newly formed patches of rust further restrict the access of oxygen to these parts of the surface. The development of focal corrosion is assisted by the poor quality (porosity) of the paint used on the hydroelectric station.

Our laboratory investigations showed that corrosion of the metal surface could be completely prevented by the use of zinc protectors when the ratio of the surfaces of protector and steel was 1:50. Several coats of bituminous paint No. 177 give better protection of the metal surface against corrosion. If the number of pores in the coating is large and the structure comes in contact with the corrosive medium, the corrosion of st. 3 steel samples is also observed in distilled water (containing no iron bacteria), the only difference being that the corrosion is much slower.

There are no grounds for attributing the primary role in pitting corrosion of metal in these conditions to iron bacteria.

All the facts noted here confirm the view that electrochemical corrosion occurs in this case. As regards the role of iron bacteria in the corrosion of metal structures we can note the following: Observations showed that the excrescent concretions of corrosion products were formed over the pores and at other points where the protective film was damaged. If the conditions did not favor the operation of local cells, a solid layer of corrosion products was formed on the unprotected metal surface. When local potential differences act for a long period and a certain amount of iron is dissolved the iron bacteria attack this iron and become involved in the formation of concretions. The latter cover part of the metal surface and limit the access of oxygen to these parts. On such parts (which act as anodes in the local cells) the corrosion process advances deep into the metal. The development of pitting corrosion is assisted by the pH reduction due to the vital activity of the iron bacteria. Microbiological investigations carried out in connection with this work showed that in those media in which iron bacteria developed the pH of the medium was lowered by one unit after three weeks of bacterial growth and development. The local change in pH under the excrescent concretions must certainly lead to an increase in the rate of corrosion.

Besides this, iron bacteria and sulfate-reducing bacteria (sulfur bacteria are present in Kama water) are biological depolarizers. When these are present electrochemical corrosion is more vigorous.

The authors regard it as a pleasant duty to express their thanks for advice and assistance in this work to docents Yu. G. Mitrofanova, K. N. Bel'tyukova and K. F. Filippova.

SUMMARY

The corrosive medium to which metal structures of the Kama Hydroelectric Station are exposed was studied. The water had a high chloride and sulfate content and the composition of the water (during the ice cover) varied with depth. Chlorides predominated at the surface and sulfates at the bottom. The oxygen content of the water varied with depth.

Laboratory and field tests on corrosion of specimens of st. 3 steel showed that the intense corrosion of metal structures was due to vigorous electrochemical processes.

Kama water contained the following iron bacteria—Leptothrix (two species), Crenothrix, and Spirophylum ferrugineum—which take part in the formation of concretions. Iron bacteria and sulfate-reducing bacteria, which are biological depolarizers, stimulate electrochemical processes.

LITERATURE CITED

1. V. O. Kalinenko, 1946, Mikrobiologiya 15, 1.
2. V. O. Kalinenko, 1949, Mikrobiologiya 28, 6.
3. V. O. Kalinenko, 1956, Mikrobiologiya 25, 3.

MICROBIAL INDICATORS OF ORGANIC POLLUTION OF WATERS BY INDUSTRIAL EFFLUENTS*

I. Filamentous Bacteria of the Genus Cladothrix (Methods, Morphology, and Relation to Physical Environmental Factors)

A. S. Razumov *

Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 515-524, May-June, 1961
Original article submitted January 9, 1961

The system of saprobic organisms and the assessment of the degree of pollution of natural waters now require a considerable reexamination in view of the great preponderance of industrial effluents over domestic sewage and the increasing diversity of their qualitative composition.

The list of pollution indicators, which now numbers more than 2500 plant and animal organisms, is still tending to increase. Yet, apart from a few exceptions, the physiological, ecological and biological features which decide their position in the system of saprobic organisms have not yet been clarified.

Moreover, it has now become imperative to improve the biological analysis used for the assessment of processes associated with the pollution of waters and their natural self-purification.

In view of the above, systematic measures will have to be taken; 1) to provide more precise knowledge of the physiology, biology, and ecology of the principal indicator organisms developing most abundantly in waters and hence to reveal what conditions, particularly what specific features of the effluents or the biochemical processes occurring in them, are indicated by these organisms; 2) to assess the role of these abundant organisms in the self-purification of waters; 3) to discover the effect of external conditions on these abundant organisms so that their indicator significance might be correctly and thoroughly specified.

The scope of the present work involved such a study of the filamentous bacteria of the genus Cladothrix.

METHODS

The work was conducted to discover the significance of filamentous bacteria as indicators and their role in natural self-purification.

Samples of filamentous bacteria were collected in waters, in industrial heat exchangers, and in the biological film and activated sludge of biological purification plants. In particular, samples were taken from the R. Moskva, R. Yauza and R. Setun', from the R. Kostromka and the industrial effluents entering it, from the R. Krasivaya Mecha and its tributary, the R. Lyubashevka, which are polluted by effluents from alcohol plants and synthetic rubber plants, the R. Volga near Yaroslavl, the R. Kan (tributary of the Yenisei), which is polluted by effluents from a hydrolysis plant, L. Kaban (in Kazan), Klyaz'ma Reservoir and certain other waters (streams, drains, sewers).

This material enabled an accurate ecological characterization of the filamentous bacteria and a precise determination of their position in the system of saprobic organisms.

The collected material was used to obtain enriched cultures of filamentous bacteria and, in several cases, pure cultures too (for instance, samples from R. Moskva, R. Volga, L. Kaban, biological film and activated sludge).

Experience showed that it was extremely difficult to obtain colonies of these bacteria by mixing the collected material directly with meat-peptone agar and plating out.

The material had to be selected from well-developed growths collected, as far as possible, immediately before the isolation of pure cultures was undertaken. Considerable difficulties arose in attempts to isolate a culture of these bacteria from long-kept samples or from aquaria. This was obviously due to the development of foreign bacteria capable of growing in association with the filamentous bacteria and even of suppressing the weak growth of the latter.

The collected material, particularly from activated sludge or biological film, had to be thoroughly freed of coarse foreign particles and washed in tap water to remove bacteria in a suspended state (microbial plankton). Washing was best performed in a weak stream of water. The filamentous bacteria were then transferred into flasks or petri dishes containing sterile water. The bacteria must not be damaged when the growths are transferred from one vessel to another. For this purpose I recom-

* This paper was completed in 1953 and is published posthumously.

TABLE 1. Characterization of Bacteria of Genus Cladothrix (from literature)

Name of organism (according to author)	Year	Author	Dimensions, μ			Presence of branches and gonidia	Temperature	Physiological characters	Ecology
			cells	including sheath	length				
Clad. dichotoma	1875	Cohn (cited by Kholodnyi)	0.3	—	—	Present, spurious	—	—	On decaying algae
" "	1882	Zopf (cited by Kolkwitz)	Up to 1.5—2.0	—	6.0	" "	—	—	Bogs, ponds, on aquatic plants, sewage
» »	1889	Schröter (")	Up to 3.0	—	6,9	» »	—	—	" "
Sph. roseus	1892	Zopf (")	Up to 2 0	—	—	» »	—	—	In flowing and polluted water
Clad. dichotoma	1894	Büsgen	2.0	—	8	—	—	—	In polluted water; not in fresh sewage
Clad. (Cohnidonum dichotoma)	1898	Mez	Up to 2.0	—	6—10	Present, spurious	—	—	In warm polluted waters
Sph. natans	1898	»	2—3	—	Short	" "	—	—	—
Clad. dichotoma	1895	Fischer	4 0	—	—	» »	—	—	—
Sph. natans (Kutzing)	1893	According to Kolkwitz	2.0	6—10	4—6	Spurious	Up to 4°		Polysaprobic and α-mesosaprobic
Sph. fluitans (Schicora)	1899	Acc. to Linde	3.0	—	6—7	»			
Clad. dichotoma	1900	Migula	2.0	—	—	»	—	—	Bog water, among decaying algae
" "	1910	Molisch	2.6			»	30—35	Assimilates nitrates, ammonium salts, mannitol, glucose, sucrose, galactose, lactose	Pits near Berlin lock
» »	1913	Linde	1—2.5	—	—				
» »	1915	Zickes	2—2.5	—	3	Gonidia motile	27—29	Liquefies gelatin, slowly assimilates nitrates, ammonium salts	On Elodea leaves
Sphaerotilus	1918	Trommsdorff	—	—	—	—	—	Assimilates nitrates, ammonium salts, carbohydrates	Effluent from cellulose factory
Sphaer. natans	1925	Eiferth	2—3	—	4—6	Branching rare	—		Polysaprobic and α-mesosaprobic
Clad. dichotoma	1925	»	Thinner than Sph.						
" "	1927	Lehmann-Neumann	1—2.5	1.5	3—8	Lophotrich	—		Natural water and sewage
Clad. (Sph. dichotomus)	1939	Bergey	1.5—2.5	2—4	—	Lophotrich, branching spurious		Liquefies gelatin slowly, assimilates mineral nitrogen	Bog water
Sph. natans	1940	Lackey a. Wattie	1—3	3—8	—		37° max	Assimilates monosaccharides and disaccharides, nitrogen of organic and inorganic compounds, pH 5.0-8.0	R. Ogaio and ewage
Sph. dichotomus	1949	Krasil'nikov	2.0	—	—	Gonidia motile	—	Liquefies gelatin	Bog water

TABLE 2. Thickness of Cladothrix Filaments from Waters and Pure Cultures

Microorganisms	Origin of culture	Cell diameter, μ		
		min.	max.	mean
Sph. (Clad.) roseus	R. Moskva, near Kamennyi Bridge	0.9	1,4	—
	The same, stained with methylene blue	1.0	1,7	1.3
Cladothrix	R. Setun'	1.0	1.5	1,3
	" "	0.6	1,7	—
	Klyaz'ma, Karabanovo	1.5	2.0	1.8
	Outflow at "Kauchuk"	0 8	1.6	1.4
	Activated aeration tank sludge	0.7	1,1	0.9
	From growths in condenser of M.G. Hydroelec. St.*	1 2	2 7	1.9
	Pure culture*	1 1	1,6	1.4
	The same, 24 hr of cultivation, 37 C	1.2	1.9	1 6
	The same, four days of cultivation, 37 C	1.2	2.3	1.8

*Cladothrix No. 21.

mend forceps or glass capillaries and not a platinum wire or loop. The capillary tip containing the bacteria filaments can be broken off into the new vessel.

For isolation of bacteria of the Sphaerotilus type the washed material can be used to inoculate meat-peptone agar diluted three to five times with "starvation" agar (i. e., prepared from tap water with no additions).

For isolation of bacteria of the Cladothrix type good results were given by a medium of the following composition (mg/liter): sucrose – 1000; KNO_3 – 500; K_2HPO_4 – 50; pH 7.3-7.6; tap water – 1000 ml.

The medium was filtered two or three times through the same cotton filter and was then poured into test tubes and sterilized. Before inoculation the medium was poured in advance (2-3 days earlier) into petri dishes to dry up the agar surface so that the growth of motile forms which develop more rapidly than the filamentous bacteria would be restricted.

For inoculation the smallest possible strands of washed growths were deposited on the surface of the medium by a platinum loop or a glass capillary sealed and bent at the very tip.

Residual water could be removed by moving the inoculum over the surface of the medium. The cultures were grown at 20-30 C. The dishes were examined periodically (in the inverted position) under the low power of the microscope. The position of colonies free of foreign bacteria was marked with India ink and such colonies were used for subcultures (after 2-7 days). Some of the colonies along with the agar were cut out by a platinum loop and transferred to the same medium for final isolation or to liquid media (composition given later).

If a pure culture could not be obtained in this way, it was better to repeat the procedure with fresh initial material than to wash previously collected samples (Razumov [1953]).

In the system of saprobic organisms Sphaerotilus natans Ktz. and Sphaerotilus roseus (Zopf [1882]) belong to the "dominant" organisms of the polysaprobic zone. Forms with a branched structure like that of Cladothrix belong to the α-mesosaprobic indicators. Filaments with frequent pseudo-dichotomous branching are designated as C. dichotoma and are regarded as indicator organisms of the β-mesosaprobic zone (Dolgov and Nikitinskii [1927]). These morphological differences of two organisms assignable to different genera are very vague and it is believed that some experience is necessary to distinguish them correctly.

It will be shown later that there is no justification for assigning these bacteria to two genera, and that frequently there are no grounds even for regarding them as separate in the genus Sphaerotilus or Cladothrix.

MORPHOLOGY OF FILAMENTOUS BACTERIA OBTAINED IN PURE CULTURES

Growth on solid media

Cladothrix colonies on agar media in petri dishes were very similar to Bacillus mycoides colonies and were rather difficult to separate without adequate experience. The bacteria spread over the surface in strands composed of several parallel filaments. At the edges of the colony the filaments coil up to form ringlets and sometimes these ringlets break off from the remainder of the colony. It was best to make subcultures from the initial plates after 5-7 days, although growth of the inoculum was frequently found on the following day. The same type of growth was observed also in streak inoculations on agar slants. The bacteria were difficult to separate from the agar, and hence subcultures from these media were better made with fragments of the cultures together with the medium. Generally the growth on agar media was rather sparse and soon ceased, and subcultures from them often gave no growth.

The cultures which I had did not liquefy gelatin or liquefied it extremely slowly. Several investigators who have had pure Cladothrix cultures have observed slow or even rapid liquefaction of gelatin by them (Table 1).

TABLE 3. Cell Width in Cladothrix (in divisions of screw ocular micrometer; one division = 0.05μ)

Origin of culture	Mean width of cells
Outflow at "Kauchuk" factory (dried and stained preparation)	15.3 – 19.7 – 24.0 – 29.3 – – 36.0
Pure culture of the same growths, two days' cultivation at 37C (1 g sucrose per liter + KNO_3)	
Stained with gentian violet (without drying)	16.0 – 20.0 – 29.6 – 30.4 – – 37.6 – 46.0

It is obvious that this property is variable and is characteristic of different varieties of Cladothrix.

Growth in liquid media

Growth was abundant in liquid media containing either organic or mineral sources of nitrogen. In favorable conditions an appreciable growth was observed in liquid medium twenty-four hours after inoculation. Subsequently there appeared a more or less abundant layer of attached filaments on the bottom and walls of the flasks. A fairly thick ring of filaments formed later and, finally, a solid film grew over the whole surface of the liquid. This type of growth made subculturing very difficult. Subculturing by pipette was most reliable, though rather inconvenient. Good results were obtained from subcultures made by a glass capillary with a sealed and short bent tip. The capillary tip with the captured material was broken off on insertion into the sterile medium. Subcultures made by a platinum wire or loop were often less successful – the filaments were difficult to detach, suffered damage, and sometimes gave no growth.

Sizes of filaments and cells

In natural conditions (in β-mesosaprobic and oligosaprobic conditions) growths of Cladothrix (filaments and tufts) on living aquatic plants, mollusk shells, and decomposing material of vegetable and animal origin are measured in millimeters or fractions of them. In polysaprobic and α-mesosaprobic conditions and in cultures with organic nutrients the filaments are very long and attain several centimeters. The filaments are invested with a sheath capable of producing slime. Preparations in India ink showed that the diameter of the filaments including the sheath is almost two or three times more than the thickness of the contained cells. In well-nourished filaments the cell boundaries are almost or completely indistinguishable without previous staining. The length of the cells varies in a very wide range. Cells several times longer than broad, and also spherical cells, occur. Over the length of a single filament the length of the cells may differ greatly, especially if the organism is in the stage of gonidium formation.

The width of the cells and filaments is less variable, and hence these data are usually given in descriptions of Cladothrix.

The range of cell width variation according to published information is given in Table 1, and that from my observations is given in Table 2.

Observations on Cladothrix from waters and from pure cultures showed that the variability of filament thickness in this organism can be taken as a reliable character of bacteria of this genus.

The diameter of the sheath can vary considerably (Table 1) and is affected by the physical state of its slime-producing surface and nutritional conditions; for instance, a slimmer sheath is observed in media with a high carbohydrate content.

More accurate measurements of the thickness of Cladothrix cells from natural growths and from pure cultures in liquid media by a screw ocular micrometer showed that the transition from minimum diameter was not smooth, but discontinuous, i. e., all sizes are apparently not equally possible.

Table 3 shows the results of 100 measurements of the diameter of Cladothrix cells obtained from growths in effluent from the "Kauchuk" rubber factory and from a pure culture of the same bacteria obtained from these growths.

Table 3 shows that not all cell diameters occurred equally often. In natural conditions the most frequent diameter was about 24 micrometer divisions (56%); thinner filaments of about 20 divisions (17%) and thicker filaments of about 30 divisions (11%) occurred less frequently, while still thicker and still thinner ones were even rarer. In pure culture filaments 24 divisions thick (21%) and 20 divisions thick (17%) occurred most often, and, in addition, cells of larger size were more numerous.

Relationships of the same kind were found in several other series of measurements, which are not given here.

The dimensions of the different categories of cells bore a definite relationship to one another, viz., the dimensions of each group differed from the previous or following one by a constant value $\sqrt[3]{2}$. For instance, if the width of one series of cells is indicated as a, the size of the next series will be $a\sqrt[3]{2}$ or $a/\sqrt[3]{2}$, and so on.

The same kind of phenomenon was observed by Novogrudskii and Kononanko [1935] in a study of Bac. mycoides. They found three categories of cells in four dif-

TABLE 4. Growth of Cladothrix at Different pH Values (estimated according to five-point system)

pH			2 Days		10 Days		Sugar	
At start	at end							
	Cl. 21	Cl. L	Cl. 21	Cl. L	Cl. 21	Cl. L	Cl. 21	Cl. L
5.2	5.2	5.2	+—	+—	0	0	+++	+++
6.3	6.1	6.5	+—	+—	+	+	+++	+++
7.0	7.0	6.9	+++++	+++++	+++++	+++++	0	trace
8.0	7.2	7.4	++++	++++	+++	+++	trace	++
9.0	7.8	7.9	+++	+++	+++	+++	*	+++

ferent strains of this bacterium, a most frequently occurring "medium" size, and also "thin" and "thick" ones, the dimensions of which were connected by the same relationships.

According to these authors, these bacterial "biotypes" differed significantly in certain characters (for instance, in rate of spore formation, resistance to heating, etc.).

They concluded that "the regular numerical relationships of the diameters of these forms of Bac. mycoides" definitely suggest that they are not autonomous, but represent a "conjugate series of forms (Uspenskii [1943]) derived from one another by a mechanism unknown to us."

In a discussion of Novogrudskii's work [1935], Mishustin [1947] confirmed the existence of Bac. mycoides forms with different cell width, but believed that "within the limits of one variant the existence of cultures with different cell width is not established. This character only serves to distinguish cultures assignable to different variants." The ratio of the diamaters in this microbe was different—it was close to 1.10-1.15.

Parallel measurements of the same filaments showed that the individual measurements were usually accurate to ±2 divisions of the ocular micrometer, i. e., to about 0.2 μ.

Mishustin indicated the distorting effect of drying and staining of the bacterial preparations as one source of error in the measurements. In this case the presence of a sheath of Cladothrix protected the cells from contraction in the transverse direction. Only their length was altered greatly with this treatment.

I made some measurements on unstained preparations and preparations stained without drying (Table 3, pure culture experiment).

For a precise determination it would possibly have been better to increase the number of measurements to several hundreds.

Observations showed that in material taken from natural conditions filaments of different size can produce gonidia. As regards my pure cultures, they were not all obtained from a single cell (from a gonidium, for instance), but from a group of filaments, i. e., the different dimensions of filament could be preserved in laboratory conditions, too. Culture conditions could also lead to the formation of thinner and thicker filaments. For instance, cells of large diameter were observed in media containing sucrose and in those filaments with iron deposited in the sheaths.

If the transition of Cladothrix filaments of different thickness from one cateogory to another was an easy process, there was apparently no reason for the presence of different forms in a homogeneous medium.

If the forms have a certain stability and, on the other hand, are due to the effect of environmental conditions, as Uspenskii found in the case of Spirogyra (Uspenskii [1934]), then the diameters of the filaments of this bacterium could be used to determine the effect of external factors on it in the case of relationships difficult to detect by other means.

RELATION TO TEMPERATURE

Cladothrix occurs in natural consitions (in water) and may form abundant growths both in summer and in winter (i. e., at +4C and 0C). In industrial effluents of suitable chemical composition and in industrial heat exchangers (saltpans, condensors, etc.), Cladothrix occurs and even grows very abundantly at 37 C, but not above 40 C (Razumov [1953a, b]).

In laboratory conditions and in pure cultures all the investigated Cladothrix strains from waters, activated sludge, biological film, or industrial appliances developed well at +24C, +30C, and up to +37C. Sometimes at 37C inoculation of flasks containing liquid nutrient resulted in an abundant formation of flocs of filaments and a film of them on the surface in less than 24 hr.

At such a high temperature growth and utilization of the medium was very rapid, thus leading to the exhaustion of the medium and weakening of the culture (poor "subculturability"), and hence the experiments on the physiology of Cladothrix were conducted at 20 or 30 C. Growing cultures had to be kept at lower temperature.

As regards Sphaerotilus in the proposed new concept of this category of filamentous bacteria as of the type studied by Brif [1936] and Smith [1934], the temperature for optimum development is lower, and at 37 C they die.

RELATION TO pH

Experiments to reveal the relation of Cladothrix to the pH were conducted with nutrient medium of the following composition (mg/liter): peptone—500, potassium phosphates—250, glucose—500; tap water—1000 ml.

TABLE 5. Effect of Dissolved Oxygen on Development of Cladothrix

Culture	Times of observation, days	
	1	20
Cl. L	Film on surface	Film was a little thicker
The same under layer of liquid paraffin	Loose flocks within medium	No changes
Cl. 21	Film on surface	Growth stopped
The same under layer of liquid paraffin	Loose flocs within medium	No changes

The medium was poured in 50 ml batches into 100 flasks. The required pH in the range 5.2-8.0 was created with a buffer mixture of mono- and dibasic potassium phosphate (pH 9.0) by alkalization with dibasic phosphate. Two Cladothrix strains isolated from growths in the R. Moskva (Cl. 21 and Cl. L) were used in the experiments. The cultures were grown in an incubator at 37C. The results of the experiment are given in Table 4, which shows that the optimum pH was in the range 7.0-8.0. In subsequent experiments and in media for maintaining the cultures the pH was adjusted to 7.3-7.6. A qualitative reaction for sugar (with Fehling's reagent) showed that glucose consumption was most rapid in this pH range. In the same cultures reduction of nitrates to nitrites was noted (qualitative reaction with iodine–zinc–starch by Trommsdorf's method). This result and further experiments showed that Cladothrix does not produce acids in media containing carbohydrates and peptone. Sphaerotilus, according to Brif's data [1936], has an optimum of development in the same pH range (7.4-8.2).

In experiments with sewage liquid and artificial nutrient medium containing (besides mineral salts) peptone (600 mg/liter), urea (200 mg/liter), meat extract (200 mg/liter) and glucose (1000 mg/liter), Ruchhoft and Kachmar [1941] found that a pure culture obtained from Lackey and which he called S. natans had an optimum of development at pH 6.6-9.0 and a temperature optimum at 30 C, but grew well at 37 C also. As regards the last index, it is more like Cladothrix than Sphaerotilus.

RELATION TO OXYGEN

In natural conditions Cladothrix occurs when the oxygen content of the water is high. Abundant growths of this filamentous bacterium can be found on submerged water plants, bridge piles, objects washed by water, on dam spillways, mill wheels, and in industrial heat-exchange appliances.

In liquid media Cladothrix usually grows over the bottom and walls of flasks and tubes or forms a dense mat of filaments on the surface.

According to Ruchhoft and Kachmar's observations [1941], their Sphaerotilus culture developed well when the oxygen content was 6 mg/liter, but growth also occurred at 0.1-2.0 mg O_2/liter.

Table 5 shows the results of an experiment with Cladothrix cultures (Cl. 21 and Cl. L) when access of air was restricted. For this purpose I conducted an experiment in which the liquid medium (1000 mg sucrose per liter and 500 mg of potassium nitrate per liter) was inoculated with pure bacterial cultures. In some of the tubes a 2 mm thick layer of sterile liquid paraffin was deposited on the top of the nutrient. The cultures were grown at 37 C.

The growth of the cultures was more abundant in flasks which were less than half full of medium, and was even more abundant in the same media in petri dishes.

Thus, these and many other observations of a similar kind showed that Cladothrix is a definite aerobic organism.

SUMMARY

The taxonomy of the filamentous bacteria used as indicators of pollution of water is imperfect and confused Sphaerotilus natans (Ktz.) and Sphaerotilus roseus (Zopf) are classed as organisms of the polysaprobic zone; the same species, but with branches as in Cladothrix, are classed as α-mesosaprobic indicators, while filaments with frequent spurious dichotomic branching are assigned to Cladothrix dichotoma and are regarded as indicator organisms of the β-mesosaprobic zone.

For a correct assessment of the pollution zones of waters and the operation of biological purification plants a thorough study of the most important indicator organisms as regards morphology, taxonomy, physiology, ecology, and variability under environmental conditions is essential.

Such a study was made of one of the water pollution indicators–filamentous bacteria of the genus Cladothrix. For a precise ecological characterization of filamentous bacteria pure cultures were isolated from growths in waters, from rivers polluted by various industrial effluents, and from the biological film and activated sludge of biological purification plants.

In the first part of this paper an account was given of methods of isolating pure Cladothrix cultures and of the resulting morphological and physiological data.

1. The cultures give a rather scanty growth on agar media. The colonies are very like those of Bacillus mycoides. Growth is more abundant in liquid media. The growth consists of a layer of filaments attached to the bottom and walls of the flask and later a dense film develops on the liquid surface.

2. In natural β-mesosaprobic and oligosaprobic conditions the filaments measure a few millimeters or fractions of millimeters. In the α-mesosaprobic zone and in cultures containing organic matter the filaments measure

several centimeters. The length and breadth of the filaments vary with the cultivation conditions. In sucrose-containing media the diameter of the filaments is greater.

3. The irregularity of cell thickness is a characteristic feature of Cladothrix.

4. Cladothrix filaments are invested with a slime-producing sheath. In natural conditions they form gonidia.

5. Cladothrix can develop at temperature +4 to +37C. The optimal pH for its development is 7.0-8.0. It is a definite aerobic organism.

LITERATURE CITED

1. R. I. Brif, Mikrobiologiya 5, 505 (1936).
2. G. I. Dolgov and Ya. Ya. Nikitinskii, Coll.: Standard Methods for Investigation of Drinking Water and Sewage [in Russian](Izd. post. byuro Vses. vodopr. i san. tekhn. s'ezdov, 1927).
3. N. A. Krasil'nikov, Key to Bacteria [in Russian](Izd. AN SSSR, 1949).
4. E. N. Mishustin, Ecological and Geographical Variability of Soil Microorganisms [in Russian](Izd. AN SSSR, 1947).
5. D. Novogrudskii and E. Kononenko, Mikrobiologiya 4, 4 (1935).
6. A. S. Razumov, Biological Growths in Drinking and Industrial Water Supplies and Measures for their Control [in Russian](Izd. In-ta VODGEO, 1953a).
7. A. S. Razumov, Dissertation: Microbial Plankton [in Russian](OBN AN SSSR, 1953b).
8. E. E. Uspenskii, Mikrobiologiya 3, 2 and 4 (1934).
9. Bergey's Manual of Determinative Bacteriology (1939) 5th Ed., pp. 881-886.
10. M. Büsgen, 1894, Berl. Dtsch. Bot. Ges. 12, H. 6, 147.
11. F. Cohn, 1875, Beitr. Z. Biol. Pfl. 1, 185.
12. Fiferth and Scheinichen, 1925, Einfachste Formen des Tier-u. Pflanzenreiches. Berlin.
13. A. Fischer, 1895, Vorlesungen über Bakterien.
14. Y. Lackey and E. Wattie, 1940, Sewage Works. J. 12, No. 4, 669.
15. K. Lehmann and R. Neumann, 1927, Bakteriologie insbesondere bakteriologische Diagnostik, II, III Aufl. 514 München.
16. P. Linde, 1913, Ztbl. Bakt. Abt. II, 39, No. 15/17.
17. P. Linde, 1913, Ztbl. Bakt. Abt. II, 39, No. 15/17.
18. C. Mez, 1898, Mikroskopische Wasseranalyse, Berlin.
19. W. Migula, 1900, System der Bakterien. II, 1033; Sphaer. natans var. Cladothrix.
20. H. Molisch, 1910, Die Eisenbakterien. Jena.
21. C. Ruchhoft and J. Kachmar, 1941, Sewage Works J. XIII, 1, 3.
22. J. Smith, 1934, Sewage works. J. VI. 6, November.
23. K. Trommsdorff, 1918, Ztbl. Bakt. Abt. II, 48, 62.
24. H. Zisches, 1915, Ztbl. Bakt. Abt. II, 43, 19/24.
25. W. Zopf, 1882, Beiträge zur Physiol. u. Morphol. Niedereorganismen, H. 2, 32.

INTENSIFICATION OF BIOLOGICAL PURIFICATION OF INDUSTRIAL WASTES

II. Microbiological Characterization of Activated Sludges Used to Purify Effluents Containing Hydrogen Sulfide

Ts. I. Rogovskaya and M. F. Lazareva

All-Union Research Institute of Water Supply, Sewerage, Hydrotechnical Construction and Engineering Hydrogeology, Moscow

Translated from Mikrobiologiya, Vol. 30, No. 3, pp. 525-529, May-June, 1961
Original article submitted May 6, 1960

Underground waters of sulfur fields contain up to 200 mg of hydrogen sulfide per liter. In view of the fact that the quantity of these waters amounts to tens of thousands of cubic meters per day, the discharge of such effluents into a natural water is impermissible owing to their toxic effects on the flora and fauna and their alteration of the oxygen conditions.

Physical and chemical methods of purification can greatly reduce the concentration of hydrogen sulfide, but they do not ensure its complete oxidation without the use of a great quantity of reagents.

Sulfur and its compounds can be oxidized by sulfur bacteria and thiobacteria, which occur widely in nature. These microorganisms are represented by various species with distinctive morphological and biochemical characters

Pleshakov [1956], who devised a method of freeing artesian water of hydrogen sulfide in concentration up to 45 mg/liter, was the first to employ a biochemical method for this purpose. Slag-filled filters with natural and artificial aeration were used as the purifying plant. The author noted that biochemical oxidation of hydrogen sulfide did not begin till the appropriate microflora had developed in the plant. The author did not investigate the microflora of the biological film. In a study of the effect of certain reduced sulfur compounds (sulfides, sulfites, thiosulfates) on the purification of domestic water by activated sludge, Donald, Aulenbach et al. [1955] found that sulfites in concentration 500 mg/liter (with 6-hr aeration) and in concentration 1000 mg/liter (with 20-hr aeration) did not affect the normal operation of the activated sludge. Sulfide was more toxic and in concentration 100 mg/liter destroyed the activated sludge. Thiosulfate in concentration 750 mg/liter did not affect the normal purification of domestic sewage.

Kalabina, Lebedeva and Stolyarova [1960] made a study of the complete biochemical purification of artificial effluents simulating sulfur-field drainage waters containing up to 200 mg of hydrogen sufide per liter.

The work was conducted on laboratory models of a mixer aeration tank* (Fig. 1) and a biological filter. The aim of our work was to study the microflora developing in these appliances.†

METHODS OF INVESTIGATION

Activated sludges were sampled from aeration tanks purifying two effluents of different composition and concentration (Table 1).

The effluent fed into aeration tank No. 1 had a total salts content of 3 g/liter, and that fed into aeration tank No. 2 contained 15 g/liter. The compounds contained in the effluents are given in Table 1.

Biogenic elements were added to both effluents – phosphorus (in the form of K_2HPO_4) in amount 2.5 mg/liter, and nitrogen (in form of NH_4Cl) in amount 5 mg/liter. The pH was maintained at 8.0.

The microbiological composition of the activated sludges was analyzed for the physiological and systematic groups of bacteria. We also examined the sludge under the microscope. The thiobacteria, sulfate-reducing, denitrifying and putrefactive microorganisms were counted by the serial dilution method. Thiobacillus thioparus were counted in Beijerinck medium, Thiobacillus thiooxidans in Waksman medium, Thiobacillus denitrificans in Beijerinck's medium, sulfate-reducers in Tauson's medium, and denitrifiers in Gil'tai's medium. For a count of the putrefactive bacteria we used 2% meat-peptone bouillon and litmus and lead indicator paper. The numbers of saprophytic aerobic microorganisms were determined by a colony count on meat-peptone agar after incubation at 20-21 C for two and

* A series of flasks filled with cadmium acetate was used to trap the hydrogen sulfide blown out of the aeration tank.

† In this paper we deal only with the microbiological characterization of the activated sludges.

TABLE 1. Composition of Effluents Purified by Biochemical Method

Compounds	Concentration of salts, mg/liter	
	in aeration tank No. 1	in aeration tank No. 2
$CaSO_4 \cdot 2H_2O$	991	2042
$MgSO_4$	149	605
$MgCl_2$	-	1315
NaCl	225	10,755
Na_2SO_4	1315	
Na_2S from calculation for H_2S	130–140	150–200
Total quantity of salts	3000	15,000

five days. The anaerobic saprophytic forms were counted in Wilson-Blair medium. The total number of microbes was determined by Razumov's direct method [1932].

Before the microbiological investigation the activated sludge was treated by the method used in a previously published paper (Rogovskaya and Lazareva [1939]).

RESULTS OF INVESTIGATION

According to the results of microscopical examination, the sludge which purified an effluent with a solid residue of 3 g/liter (aeration tank No. 1) consisted of zooglea with long finger-like outgrowths formed by small colorless rods (Fig. 2). In the sludge flocs we found spherical sulfur-yellow formations. These were droplets of amorphous sulfur.

The sludge which purified the effluent with a solid residue of 15 g/liter (aeration tank No. 2) consisted of compact rounded zooglea formed by tiny colorless rods (Fig. 3). The sludge was sulfur-yellow. Besides the zoogleal accumulations the sludge liquid in the aeration tanks also contained small numbers of solitary bacterial cells—rods and cocci of a pink color; amoebae were rare in the sludge.

Results of the microbiological analysis of the sludges are given in Table 2.

TABLE 2. Composition of Microflora of Activated Sludges and Number of Bacteria in 1 ml of Wet Sludge

Microbes	Number of bacteria in 1 ml, millions	
	aeration tank No. 1	aeration tank No. 2
Thiobacillus thiooxidans	1000	1000
" thioparus	10	0.001
" denitrificans	0.1	0.1
Sulfate-reducers	0.001	0.001
Denitrifiers	0.001	0.001
Putrefactive, producing H_2S	0	0
" " NH_3	0.001	0
Aerobic saprophytes	0.03	0.2
Anaerobic saprophytes	0	0
Total number of bacteria counted by culturing method	1010	1000
Number of anaerobes, as percentage	0.01	0.01
Total number of bacteria counted by direct method	20 000	10 000

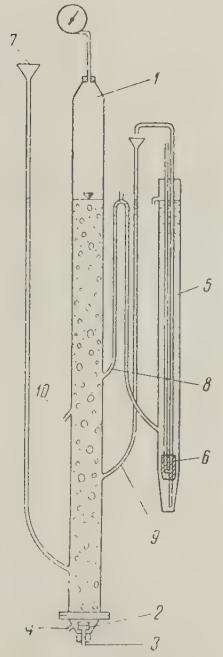


Fig. 1. Diagram of mixer aeration tank. 1) Aeration tank; 2-3) air diffuser or sparger; 4) tube for removal of activated sludge; 5) sedimentation vessel; 6) air-lift; 7) funnel for feed liquid; 8) device for transfer of sludge and water mixture from aeration tank to sedimentation vessel; 9) device for transfer of sludge from sedimentation vessel to aeration tank; 10) device for sampling of sludge liquid.

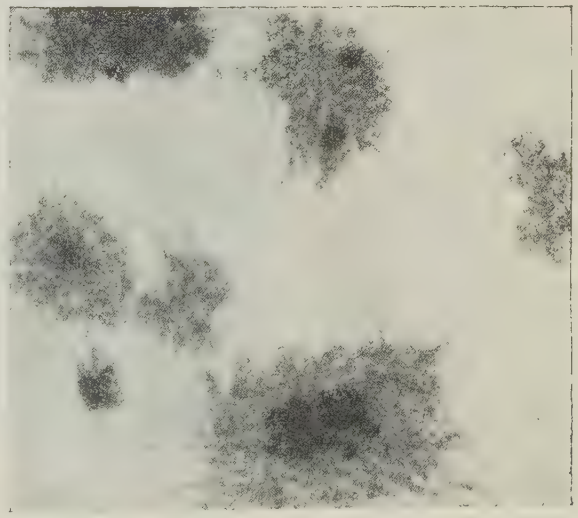


Fig. 2. Activated sludge purifying effluent with solid residue 3 g/liter.

TABLE 3. Characterization of Operation of Aeration Tanks

Indices	Aeration Tank No. 1		Aeration Tank No. 2	
	effluent		effluent	
	non-purified	purified	non-purified	purified
Load*	5 : 1	—	6 : 1	—
Amount of air, mg/m^3 liquid	18	—	20	—
Dissolved oxygen, mg/liter	0	5.5	0	2.6
Hydrogen sulfide, mg/liter**	151.3	0	173.4	0
Sulfates, mg/liter	167.4	302	395.0	538.8
pH of water	8.2	3.8	7.9	4.8
Concentration of sludge, g/liter	0.7	0.7	3.0	3.0
Sludge ash, %	7.8	7.8	6.5	6.5
Sludge index	—	93	—	13

*Load—number of volumes of liquid per volume of tank per day.

**All sulfur compounds are expressed as S.

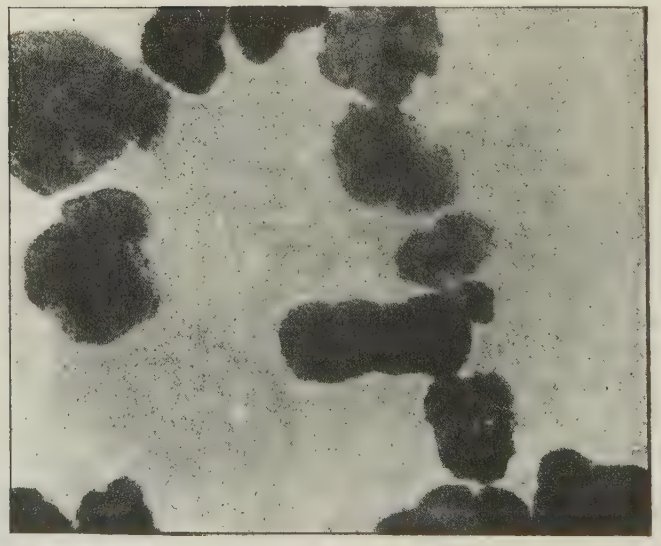


Fig. 3. Activated sludge purifying effluent with solid residue 15 g/liter.

Data on the oxidative work of the aeration tanks at the time of the microbiological investigation of the sludges are given in Table 3.‡

Thiobacteria, sulfate-reducers, denitrifiers, putrefactive, and saprophytic bacteria (aerobic and anaerobic) were found in the sludges.

The bulk of the microflora of the activated sludges consisted of microorganisms effecting the biochemical oxidation of hydrogen sulfide and other sulfur compounds.

The presence of aerobic saprophytes in sludges purifying effluents containing hydrogen sulfide in concentration 130 to 200 mg/liter can be explained by the fact that chemical analysis did not reveal any hydrogen sulfide in the aeration tanks. Its oxidation to intermediate products was presumably instantaneous. On the other hand, we know from published information (Stephenson [1951]) that some heterotrophic aerobic saprophytes (Pseudomonas aeruginosa, Pseudomonas fluorescens, Achromobacter stutzeri, etc.) can oxidize thiosulfates.

The saprophytic microflora which we isolated may also possess this property, but we did not identify them as to species.

In the aeration tanks the hydrogen sulfide was completely oxidized, mainly to sulfates. This was indicated by the increase in the sulfate content, and also by the pronounced acidification of the purified liquid to pH 3.8-4.8.

The conducted investigations showed that although the activated sludges purifying effluents containing hydrogen sulfide were similar as regards microbiological composition and number of microbes, the rate of hydrogen sulfide oxidation by them was different.

For instance, in the more mineralized water (solid residue 15 g/liter) 1 g of ashless sludge at a load rate of 6 : 1 oxidized 0.37 g of hydrogen sulfide per day. When the tank load was increased to 7 : 1 the purification was poorer.

In water with a smaller salts content (3 g/liter) 1 g of ashless sludge at load rate 5 : 1 oxidized 1.16 g of hydrogen sulfide per day and this was apparently not the limit.

The different biochemical activity of the sludges which purified the above effluents can probably be attributed to the effect of the salt composition of the liquid on the metabolism of the bacterial cells.

According to Artari [1916] and Dumesh [1935], mineral salts and the ions formed in their dissociation have a very considerable effect on the cell protoplasm and its surface layer. The electrolytes are not only used for cell construction, but also produce physical changes in the structure of living matter. They cause it to become looser or more compact, thus affecting the permeability of the surface layer of protoplasm and, consequently, the processes involved in cell metabolism.

SUMMARY

1. Thiobacteria predominate in activated sludge purifying sulfur-field drainage waters containing hydrogen sulfide.

2. Thiobacillus thiooxidans is the dominant member of the thiobacteria group in the activated sludge. Thiobacillus denitrificans, and also other anaerobic microbes, are present in smaller numbers.

3. The abundance of Thiobacillus thiooxidans in the sludge ensures complete oxidation of hydrogen sulfide to sulfuric acid in the aeration tank if sufficient oxygen is present. The purified liquid is clear and the pH is reduced from 8.3 to 3.8.

4. Anaerobes comprised 0.01% of the number of microbes (10^9) in 1 ml of wet sludge in the aeration tank.

5. According to a direct bacterial count, 1 ml of wet sludge contained $1 \cdot 10^{10} - 2 \cdot 10^{10}$ bacteria.

LITERATURE CITED

1. A. P. Artari, Studies of the Simplest Organisms in Salt Lakes [in Russian](1916).
2. M. G. Dumesh, Mikrobiologiya 4, 1, 45 (1935).
3. M. M. Kalabina, M. P. Lebedeva and L. I. Stolyarova, Biological Purification of Ground Waters from Hydrogen Sulfide [in Russian](Izd. VNII VODGEO, 1960).
4. V. D. Pleshakov, Removal of Hydrogen Sulfide from Artesian Waters [in Russian](Izd. Min-va kommun. kh-va RSFSR, Moscow, 1956).
5. A. S. Razumov, Mikrobiologiya 1, 2, 131 (1932).
6. Ts. I. Rogovskaya and M. F. Lazareva, Mikrobiologiya 28 (4): 565 (1959).
7. M. Stephenson, Bacterial Metabolism [Russian translation](IL, Moscow, 1951).
8. Donald B. Aulenbach and H. Henkelekian, Sewage and Industrial Wastes 27, No. 4 (1955).

‡The chemical analyses were performed by M. P. Lebedeva and L. I. Stolyarova.

PURIFICATION OF MICROBIAL HISTIDINE DECARBOXYLASE

S. R. Mardashev and V. V. Mamaeva

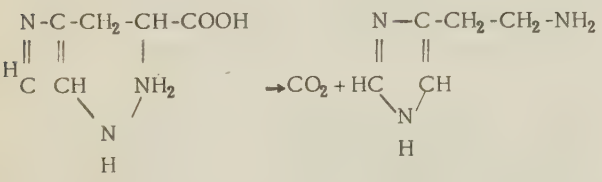
I. M. Sechenov First Moscow Medical Institute

Translated from Mikrobiologiya, Vol. 30, No. 3, pp. 530-533, May-June, 1961
Original article submitted October 19, 1960

Microbial acetone preparations containing active amino acid decarboxylases have been used successfully in analytical practice for analysis of the amino acid composition of proteins (Gale, 1946; Mardashev, 1950).

However, an essential defect in microbial preparations as reagents is their large content of extraneous mater

In 1953, our laboratory (Gladkova, Mardashev, Semina, 1953) isolated a bacterium (Micrococcus sp.) that specifically decarboxylates L-histidine:



L-histidine Histamine

Our experimental work was concerned with the treatment of the micrococcal preparation in order to purify the L-histidine decarboxylase. The bacterial acetone powder was prepared according to the method generally used in our laboratory (Mardashev, 1950).

PREPARATION OF EXTRACTS

Five to ten g dry acetone powder of micrococci were mixed with 100 ml of distilled water, 1-2 small crystals of thymol were added, and the mixture was placed in a thermostat at 37C for 24 hr. The suspension was periodically stirred.

After a day's incubation the suspension was centrifuged for 15-20 min at 3000-4000 rev/min (Semina, 1953) The supernatant liquid was transferred to a distilling flask and concentrated in a vacuum at 40C to 6-8 ml. The thickened extract was then centrifuged on a high-speed centrifuge (8000 rev/min). The clear, slightly opalescent, thick, yellow solution obtained was placed in a refrigerator (first extract).

The residual microbial preparation after the first extraction was subjected to a second and third extraction by the same method. The second and third extracts were light yellow in color, turbid or strongly opalescent, not viscous. All extracts were kept in the refrigerator. Usually, when kept in the cold, a precipitate formed, from the first extract especially, which was separated by centrifugation and discarded (enzyme activity in this precipitate was entirely nil).

TREATMENT OF THE EXTRACTS WITH ACETONE

To 6 ml concentrated extract 3 ml previously cooled acetone was carefully added drop by drop (from a burette) to a final concentration of the acetone of 33%. As the acetone was added, a precipitate formed, which increased on standing in the refrigerator. After several days of standing in the cold, the precipitate was separated by centrifugation and washed successively with 33% acetone, anhydrous acetone, a mixture of acetone and ether (1:1) and finally ether. After each washing the precipitate was separated by centrifugation and as quickly as possible taken up in the succeeding portion of wash liquid. Finally, the precipitate was placed in a vacuum desiccator over paraffin and calcium chloride (first precipitation).

To the supernatant liquid from the first precipitation, 3 ml more acetone was added, that is, to a final concentration of 50% acetone; the suspension was placed for several days in the refrigerator and finally centrifuged. The precipitate was washed and dried by the methods described above (second precipitation).

To the supernatant liquid after the second precipitation, 2 ml more acetone was added and the process repeated as described above (third precipitation).

All the precipitates obtained were examined under the microscope. This examination revealed that in addition to amorphous masses, crystals of various forms sometimes separated out, which could easily be isolated from the amorphous masses; they were dried and their enzymatic activity determined. It was shown that these crystals were completely devoid of decarboxylase activity.

Among the crystals of various forms, the precipitates also contained crystals in the form of regular hexagons. These crystals could not successfully be separated from the amorphous mass. However, if different centrifuge speeds were used, it was possible to obtain such precipitates which clearly contained large quantities of the hexagon crystals, and precipitates with fewer crystals. The precipitates were collected, washed, and analyzed for the presence of decarboxylase. In the majority of cases

TABLE 1. Decarboxylase Activity of Preparations Obtained by Acetone Precipitation of Water Extracts from a Micrococcus (In a 3 ml sample, there was 1 mg preparation and 1.74 ml histidine monohydrochloride. A and B—independent experiments; in A, CO_2 determined after 10 min; in B, after 15 min)

Preparation	Original powder		First extract						Second extract						Third extract			
	A	B	precipitation						precipitation						precipitation			
			1		2		3		1		2		3		1		2	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Amount of preparation obtained, mg	5000	5000	50	43	26	16	15	17	9	43	10	12	2	2.5	13	8	6	4
CO_2, μ liters	26	9	0	25	170	173	—	161	0	134	115	36	—	0	0	131	—	0
Increase in activity	—	—	—	2.5	6.5	19	—	18	—	15	4	4	—	—	—	15	—	0

TABLE 2. Acetone Reprecipitation of Purified Histidine Decarboxylase

Preparation	CO_2, μ liters after 10 min	after 60 min
Original: 1 mg	142	153
Precipitate I (30% acetone):		
0.5 mg	15	53
0.25 »	8	32
Precipitate II (50% acetone):		
0,25 mg	163	174
0.125 »	90	171
0,063 »	52	176

TABLE 3. Adsorption of L-Histidine Decarboxylase on Calcium Phosphate Gel and Elution with Phosphate Buffer, pH 8.0

Solution investigated (1 ml)	CO_2, μ liters at 70 min	Nitrogen, mg/1 ml solution	Specific activity	Increase in activity
Extract before adsorption	176.5	0.346	510	—
Extract after adsorption	0	0.158	—	—
Eluate 1	165.7	0.111	1493	3 times
Eluate 2	52.8	0.008	6600	13 times
Eluate 3	0	0	—	—
Adsorbent after three elutions	0	0.04	—	—

the presence of hexagon crystals accompanied high decarboxylase activity.

The decarcoxylase activity was checked in a Warburg apparatus. One mg of enzyme preparation and 1.74 ml of substrate (histidine monohydrochloride) were added to each flask. The decarboxylase reaction took place in acetate buffer solution at pH 4.5 and 37C. The total volume of liquid was 3 ml.

Data on the acetone-precipitated enzyme and decarboxylase activity in the samples obtained are summarized in Table 1.

From the data presented in Table 1, it is evident that activity in our experiments was increased a maximum of 18-19 times.

The active preparations of histidine decarboxylase obtained by acetone preparation (30-50%) were subjected to a second reprecipitation with acetone after they were dissolved in water. Data from one of these experiments are summarized in Table 2, from which it follows that a second reprecipitation results in highly active preparations.

However, an attempt at further purification of these preparations by means of reprecipitation in acetone led to unexpected results. They showed that if such highly active preparations were dissolved in water and an attempt was made to carry out fractional precipitation with increasing concentrations of acetone, then no precipitation occurred at 50% or even at 60-80% acetone. From such solutions, after the acetone was driven off in vacuum, the active decarboxylase was successfully retrieved by salting out with ammonium sulfate. The most active fraction was precipitated by 0.75% saturated ammonium sulfate.

ADSORPTION OF L-HISTIDINE DECARBOXYLASE ON CALCIUM PHOSPHATE GEL

The calcium phosphate gel was prepared by the method of Keilin and Hartree (1938). Twenty-five ml of a suspension of calcium phosphate gel (1 ml suspension contained 7.9 mg dry substance) was centrifuged for 5 min. The precipitate was stirred at room temperature for 30 min with 25 ml of the water extract of micrococci and again centrifuged for 5 min.

The disappearance of enzyme activity from extracts treated with adsorbent served as a measure of the adsorption on the calcium phosphate gel.

TABLE 4. Adsorption of L-Histidine Decarboxylase on Calcium Phosphate Gel and Elution with Phosphate Buffer, pH 8.0

Test solution (1 ml)	CO_2, μ liters after 60 min	Nitrogen activity	Specific activity	Increase in activity
Extract before adsorption	174.0	0.794	219	—
Extract after adsorption	0	0.514	—	—
Eluate 1	137.0	0.187	732.7	3 times
Eluate 2	15.2	0.0127	1200	5.5 times
Eluate 3	0	0	—	—

ELUTION OF L-HISTIDINE DECARBOXYLASE FROM CALCIUM PHOSPHATE GEL

The calcium phosphate gel precipitate, having adsorbed the L-histidine decarboxylase, was periodically stirred at room temperature for 30 min with 25 ml of a solution of phosphate buffer, pH 8.0, and centrifuged for 5 min. The treatment was repeated 3 times.

The eluate was analyzed for decarboxylase activity.

Tables 3 and 4 illustrate the adsorption of L-histidine decarboxylase on calcium phosphate gel, its elution, the content of nitrogen and the specific activity calculated on the basis of 1 mg nitrogen.

These data indicate that extracts treated with calcium phosphate gel are completely without enzyme activity, but contain 45-60% of the nitrogen in the original extract. The basic decarboxylase activity is located in the first eluate. The specific activity as a result of such treatment is increased three-fold in the first eluate and 5-13-fold in the second.

The adsorbent treated three times with phosphate buffer, pH 8.0, does not have, or has only slight, decarboxylase activity and contains 6-11% of the nitrogen of the original extract.

An attempt to purify the histidine decarboxylase by means of carboxymethylcellulose (Ellis and Simpson, 1956) was unsuccessful.

SUMMARY

1. By extraction of acetone preparations of a Micrococcus sp. with water, L-histidine decarboxylase could be transferred into solution, from which it was precipitated with acetone.

2. The precipitates having high decarboxylase activity usually contained regular hexagonal crystals.

3. The greatest increase in activity of the purified acetone preparations in comparison with the original was 18-19 times on a dry weight basis.

4. L-histidine decarboxylase was adsorbed on calcium phosphate gel and eluted with phosphate buffer, pH 8.0. As a result of such purification the specific activity increased 5-13 times.

LITERATURE CITED

1. V. N. Gladkova, S. R. Mardashev and L. A. Semina, Mikrobioligiya 22, (2), 141 (1953).
2. S. R. Mardashev, Uspekhi Biol. Khim. 1, 281 (1950).
3. L. A. Semina, Dissertation: Microbial Decarboxylation of L-Histidine, First Moscow Order of Lenin Medical Institute, 1953.
4. S. Ellis and M. E. Simpson, J. Biol. Chem. 220, 939 (1956).
5. E. F. Gale, Adv. Enzymol. 6, 1 (1946).
6. D. Keilin and E. F. Hartree, Proc. Roy. Soc. 124, 397 (1938).

PRODUCTION OF PURIFIED ENZYME PREPARATIONS FROM THE SURFACE OF MOLD CULTURES

R. V. Feniksova and G. A. Molodova

A. N. Bakh Institute of Biochemistry, Academy of Sciences, USSR
Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 534-539, May-June, 1961
Original article submitted July 22, 1960

The amylolytic enzyme complex of molds, by its nature, includes several enzymes the joint action of which comes from the full hydrolysis of starch to fermented sugars.

According to Feniksova [1956] these enzymes in a culture of Aspergillus oryzae are at least three: alpha-amylase, dextrinase, and maltase. Several different names are given in the literature for the latter enzyme: glucamylase, amyloglucosydase, and gamma-amylase.

The group of microorganisms of which A. oryzae is the most characteristic representative includes a large number of alpha-amylases, manifests relatively high activity with effect on end dextrin (dextrinase) and produces few maltases. The aspergilli, in which Aspergillus niger, Aspergillus awamori and others are found, and also some species of Rhizopus, on the other hand, show high maltase activity with relatively weak alpha-amylase activity.

The presence of a large number of enzymes in molds makes an explanation of the mechanism and speed of starch hydrolysis difficult. We do not have coordinated data on precisely which component of the amylolytic complex the fullness and speed of the starch break down depends.

The output of alcohol in the starch saccharification of the molds A. oryzae and A. niger is the same, though they differ sharply in the relationship of the enzymes of the amylolytic complex [Feniksova, 1953].

The saccharification of starch through the use of unpurified enzyme preparations does not make it possible to judge the specific action of each of them. In connection with this many investigators have experimented on the production of individual enzymes and the final stage of purification and isolation – the production of these enzymes in crystalline form.

In a number of instances amylase was produced from A. oryzae by Caldwell and others [Caldwell, Chester, Doebling and Voz, 1945] by means of repeated precipitation of enzymes from water extracts by ammonium sulfate.

Fischer and Montmollin [1951] produced alpha-amylase from A. oryzae by fractional precipitation with acetone and ammonium sulfate.

By using precipitation in connection with adsorption Underkofler and Roy [1951] separated amylase, dextrinase, and maltase in a deep-seated culture of A. oryzae, during which dextrinase was adsorbed by bentonite and in which mercuric chloride was used for the break down of alpha-amylase.

In a number of cases maltase was produced from a deep culture of A. niger by these authors [Roy, Underkofler, 1951].

In a culture of Rhizopus delemar an enzyme was found which produced glucose from action on starch. Phillips and Caldwell [1951 a, b] studied the action of this enzyme in different substrates. The authors performed the work by purifying a preparation of enzymes from Rhizopus delemar and releasing glucamylase from other enzymes. It was determined that the glucamylase itself possessed maltase hydrolytic activity and starch and maltose. For the liberation of alpha-amylase an enzyme solution was aged at pH 2.4 and 5C for nine days. Kerr, Cleveland and Kalzback [1951] showed that the amylase of A. niger is more stable toward acid. The authors succeeded in tracing the mechanism of the hydrolysis of amylase and amylopectin preparations from A. niger in the presence and absence of alpha-amylase and in determining a different mechanism of starch separation from amyloglucosydase (glucamylase) and alpha-amylase.

One of the serious difficulties in purifying enzyme solutions is freeing them from pigments.

Fischer and Stein [1954] verified the entire series of methods for decolorization of enzymes. Thus, the filtering of dilute solutions through diatomaceous earth may purify the amylase, but this procedure is practically inapplicable with the majority of dilute solutions. Best results are gained through adsorption of the colored substances in barium oxide, oxalates, and phosphates.

Nevertheless, in spite of the difficulty of isolating and purifying enzymes, this method is the only accurate one which will make it possible to produce individual enzymes, to study them properly and to examine their mechanisms of action. Our problem is the production of preparations with maximal enzyme activity and the selection of the most effective methods for purification.

EXPERIMENTAL

During the last year the All-Union Science Research Institute of Alcoholic Industries has been conducting

work on the production of enzyme preparations from mold cultures. The method worked out is one of isolation of complex preparations from a surface culture in siftings, and also one of fractional separation of amylase and protease.

So far as research with mold cultures cultivated in shavings is concerned, in aqueous extracts not only enzymes are produced but also many soluble impurities, the basic difficulty being to get rid of the impurities and at the same time to keep the enzymes in active condition.

In the literature it is reported that salting out by ammonium sulfate may be the final stage of the isolation of enzyme preparations. Nevertheless, when the enzyme precipitate is isolated from the mother liquor it usually contains a large quantity of salts (90% and more) and, in our opinion, may not be considered the final product. Under some conditions preparations can not be used in manufacturing—in the confectionery industry, in the wine industry, in manufacturing liquor, etc., for example.

Most noticeably we observed that enzyme preparations produced in such a manner from malt extracts lost their activity very quickly under storage.

We therefore considered it necessary to work out a method of purifying enzyme preparations which would be acceptable for practical purposes and which would produce preparations with maximal activity.

METHODS

Preparations of enzymes isolated from mold cultures by precipitation with ammonium sulfate served as the raw material for the concentration and purification of enzymes of the amylolytic complex in the majority of experiments.

General purification techniques follow: 1) extraction of an enzyme solution; 2) dialysis; 3) treatment with lead acetate; 4) decolorization with barium sulfate at the moment of its generation in solution; 5) salting out with ammonium sulfate; 6) production of the enzyme in powder form (a precipitate by alcohol or acetone, and also desiccation in a spray desiccator).

The enzyme activity of the amylolytic complex—amylase, dextrinase, and maltase—was determined at all stages of the purification by methods used in the laboratory [Feniksova, 1956].

RESULTS

One of the methods of freeing an enzyme solution from dark-colored materials is its treatment with lead acetate.

The work requires great care, for the lead acetate, even in comparatively small quantities, inactivates the enzyme. Therefore, for each enzyme solution, it is necessary to determine by tests the fullness of saturation by lead acetate and the degree of change in enzyme activity.

The effect of the use of lead acetate in the purification of a preparation produced by precipitation with ammonium sulfate from a surface culture of A. oryzae was studied by us in two variants: 1) the treatment of the original solution with lead acetate with full use of filtration and precipitation from the solution of the surplus ammonium sulfate (instead of dialysis); in this case lead acetate is added in large quantities (25-50% by volume of the enzyme solution). 2) Purification of the dialyzed solution to get rid of pigments; in this a very small amount of lead acetate produced a considerable degree of purification, and the solution changed from dark cinnamon, almost black, to a light yellow.

For a comparative test of purification of amylase preparations by the use of lead acetate, a precipitate of enzyme produced by salting out with ammonium sulfate was used. The precipitate was dissolved in water and the solution divided in two equal parts (Table 1).

The first part (Test 1) was treated with lead acetate after a long dialysis, and, instead of dialysis, 50% lead acetate was added to the second part of the enzyme solution (Test 2).

Both solutions were then dialyzed and salted out. The enzymes from the purified solution of Test 1 were precipitated by four parts alcohol by volume at 5C, and the solution of the second test was desiccated in a spray desiccator.

A shortened purification, used in Test 2 (without dialysis in the first and last stages), gave the best results, both in activity per gram in AA units (amylolytic activity, and in yield of the preparation. At all stages of purification a marked loss of activity was observed, and in terms of yield of amylase the quantity amounted to only 10-24% of the material used in the test.

These tests turned out better when we raised the amylase activity by increasing the 300 units/g in the original preparation to 2,000-2,600 units/g, i.e., 7-8 times.

A variant of purification used in Test 2 was the repetition with large quantities of a more impure preparation.

In order to avoid loss in desiccation of the enzyme solution in the spray desiccator—where a part of the preparation is lost in the tubes and other parts of the apparatus—acetone was used for isolation of a dry preparation. Furthermore, we detected in this test a quantitative change in all enzymes of the amylolytic complex. In Table 2 an analysis of the preparation produced in comparison with the original material is given.

A complex enzyme purification in a variant of Test 2 produced an increase in the activity of all three enzymes, and to a more significant degree than in the previous test. The amylase yield in the preparation also rose from 24 to 40%.

As has been noted, one of the major difficulties in working with preparations from surface cultures is getting rid of the pigments which give the preparation a dark col-

TABLE 1. Change in Amylase Activity (AA) in the Process of Purification with Lead Acetate

Purification stage	Amylase activity, units			
	test 1		test 2	
	total activity	% of original	total activity	% of original
Aqueous enzyme solution	76 820	100	76 820	100
Dialysis	72 210	94	—	—
Treatment with lead acetate	60 000	80	65 000	84
Salting out with ammonium sulfate	51 000	66	51 000	66
Dialysis	42 250	55	42 250	55
Second treatment with lead acetate	30 000	40	30 000	40
Salting out with ammonium sulfate	26 000	34	—	—
Dialysis	24 000	31	—	—
Precipitation of enzymes	8 000	10	18 200	24
Yield and activity of the preparation	AA=2000 units/g Weight = 4 g		AA = 2600 units/g Weight = 7 g	

TABLE 2. Activity of Amylolytic Enzymes in the Original and Purified Preparations

Preparation (g)	Enzyme activity (in units)		
	AA	DA	MA
Original —800	100	810	52
Purified —13	2400	9640	683

NOTE: AA - amylolytic activity; DA - dextrinolytic activity; MA - maltase activity.

or. Treatment of the enzyme solutions with lead acetate is not always used because of the marked inactivation of the enzymes.

We therefore tested the method of decolorization with barium sulfate, which adsorbs the pigments at the moment of its production in the enzyme solution [Fischer, Stein, 1954].

For this, specific quantities of barium acetate and ammonium sulfate, in mixture, are introduced into the enzyme solution. Optimal quantities of salts from the solution depend on the intensity of the liquid's color and are determined first in small tests, as is done with lead acetate.

The data of Table 3 show that the preparation produced in the two-stage adsorption of pigments in barium sulfate (Test 1) was less active than in the one-stage treatment with barium sulfate and lead acetate. In these tests we produced more rapid amylase activity in a 1 g preparation than in earlier tests.

A fault of this method consists in the strong dilution of the enzyme solution. Therefore, after decolorization with barium sulfate it is necessary to use salting out with ammonium sulfate and a final dialysis.

A combined purification of the enzyme solution with barium sulfate and lead acetate produced a good quality of purification and rapid amylase activity (3,400-3,700 units/g).

Investigation of the amylolytic complex of molds and the production from them of enzyme preparations led us basically to a culture of A. oryzae.

We also used a culture of Rhizopus delemar, which possesses, as is known, rapid dextrinolytic and maltase activity and slight amylase activity.

The tests were conducted in such a way that amylase inactivity was produced, verification being available in the literature on amylase that it may be inactivated at a low pH of the enzyme solution and a low temperature.

Acidification with phosphoric acid to a pH of 2.5 and aging for 24 hr at 8 C was not sufficient to inactivate the amylase, for after purification by salting out, dialysis and treatment with lead acetate a preparation with rapid amylase activity (750 units/g) was produced.

We therefore increased the aging period (pH 2.4) to five days at 5 C. In this case purification produced a preparation with very rapid dextrinase and maltase activity which contained only 40 units/g of amylase (Table 4).

With protracted treatment with acid we succeeded almost completely in inactivating the amylase. At all the stages of purification it was discovered as traces and only in the dry preparation did it comprise 40 units/g, 60-90 times less than the percentage of this enzyme in preparations from A. oryzae.

In this test, as a measure of the purification, the concentration of dextrinase and maltase was raised, and in the final preparation the activity of these enzymes in a

TABLE 3. Change in Amylase Activity in the Process of Purification with Lead Acetate and Barium Sulfate

Purification stage	Amylase activity, units			
	test 1		test 2	
	total activity	% of original	total activity	% of original
Aqueous enzyme solution (650 ml)	26 000	100	26 000	100
Decolorization with barium sulfate	23 000	90	23 000	90
Salting out with ammonium sulfate	20 000	80	20 000	80
Decolorization with barium sulfate and dialysis	18 200	70	—	—
Treatment with lead acetate	—	—	17 160	66
Salting out	16 470	63	—	—
Dialysis	16 000	62	—	—
Desiccation in a spray desiccator	AA = 3430 units/g		AA = 3700 units/g	

TABLE 4. Production of a Purified Preparation from a Mold Culture of Rhizopus delemar

Purification stage	Enzyme activity, units					
	AA		DA		MA	
	total activity	% of original	total activity	% of original	total activity	% of original
Solution of the preparation	2 800	100	150 500	100	17 630	100
Amylase inactivity	Traces	—	123 300	82	15 520	88
Salting out and dialysis	»	—	109 800	73	13 930	79
Purification with lead acetate	»	—	102 350	68	11 820	67
Dry preparation, precipitated by acetone (1.82 g)	40 units/g	2.6	26 000 units/g	31.4	3860 units/g	40

short time was higher than in preparations from A. oryzae (cf. Tables 2 and 4).

The work on production of highly purified preparations from A. oryzae and Rhizopus delemar confirmed the characteristic difference in enzyme complexes that these two genera of molds have.

SUMMARY

1. Conditions were found for the purification of enzymes from surface cultures of the molds Aspergillus oryzae and Rhizopus delemar with the production of highly active enzyme preparations.

2. Treatment of the enzyme soultion with 10% lead acetate may be used to precipitate the SO_4^{2-} ion from the solution and for the removal of dark colored impurities from the dialyzed enzyme solution.

3. The combined purification of the sulfate preparation using decolorization with barium sulfate, salting out, dialysis and clarification with lead acetate yields a preparation of A. oryzae with an amylase activity of up to 3,700 units/g.

4. With the aim of isolating amylase-inactive preparations, a preparation of R. delemar was purified, resulting in an increase of dextrinolytic activity of up to 26,000 units/g and an increase of maltase activity of up to 3,860 units/g, that is, 60-65 times greater than in the original mold culture.

LITERATURE CITED

1. R. V. Feniksova, Mikrobiologiya 22, 1 (1953).
2. R. V. Feniksova, Amylolytic Mold Enzymes from the Genus Aspergillus [in Russian](1956).
3. M. Caldwell, R. Chester, A. Doebling and G. Volz, J. Biol. Chem. 161, 361 (1945).
4. E. Fischer and A. Montmollin, Nature 168, 4275 (1951).
5. E. Fischer and E. Stein, Arch. Sci. 7, 131 (1954).
6. B. W. Kerr, F. C. Cleveland and W. V. Kalzback, J. Amer. Chem. Soc. 75, 8, 3916 (1951).
7. L. L. Phillips and M. L. Caldwell, J. Amer. Chem. Soc. 75, 8, 3359 (1951 a).
8. L. L. Phillips and M. L. Caldwell, J. Amer. Chem. Soc. 75, 8, 3563 (1951 b).
9. D. R. Roy and L. A. Underkofler, Cereal Chem. 28, 1, 72 (1951).
10. L. A. Underkofler and D. R. Roy, Cereal Chem. 28, 1, 18 (1951).

EFFECT OF WHEAT BRAN QUALITY ON THE AMYLOLYTIC ACTIVITY OF ENZYME PREPARATIONS

T. I. Trainina, D. B. Lifshits, and E. Ya. Kalashnikov

Ukranian Scientific Research Institute for the Food Industry, Kharkov

Translated from Mikrobiologiya, Vol. 30, No. 3, pp. 540-544, May-June, 1961
Original article submitted August 1, 1959

Wheat bran is the most favorable culture medium for the growth of molds in the production of amylolytic and proteolytic enzymes.

The use of wheat bran for this purpose was proposed by Takamine [1914], and subsequently the advantages of its use were confirmed by a number of authors [Oparin and Yal'tsev, 1929; Feniksova et al., 1954; Harada, 1931; Oschima, 1923; Underkofler et al., 1939; Wallerstein, 1939]. We also came to such a conclusion on the basis of the test results of several years' investigations of many natural and synthetic media and of various strains of Aspergillus oryzae [Kalashnikov and Lifshits, 1946].

The fact that wheat flour from a single milling containing all parts of the wheat seed and used as the nutrient medium for the accumulation of enzymes gave way significantly to wheat bran deserves notice. Feniksova, Kinzburskaya and Shilova [1954] have explained the positive quality of wheat bran by the favorable starch-nitrogen relationship in it.

In the literature, as is known, data for the setting up of special requirements for wheat brans used in the production of enzymes, other than coarse milling and lack of a stale odor, are not given. For this reason bran produced only in accordance with the requirements of government regulation 7169-54 is being used in the enzyme plants in operation. In the past, however, we encountered individual lots of bran, which, if used, led to appreciable reduction in the amylolytic activity of the preparations made. Laboratory inspection of bran from these lots by sifting the A. oryzae mold from them showed that the saccharification capacity, as in production, was 40-60 SC (saccharification capacity) units less than the controls in good bran. To clarify the reasons for such an effect we sorted out samples of individual lots of bran from the Kharkov, L'vov and Rostov enzyme plants which showed various activity in actual production and analyzed them by starch, total nitrogen, amino nitrogen, and ash content. Under laboratory conditions enzyme preparations were made by sifting A. oryzae (strain 476 - velvety) mold from them in the preparation of a nutrient medium, and their amylolytic activity was determined (by the method recommended by PTU-USSR 45-58 for enzyme preparations of A. oryzae).

The results of this series of tests are shown in Table 1.

An examination of the data makes it sufficiently clear that the saccharifying capacity of preparations produced under various conditions is determined by the percent of starch content in the bran. The amylolytic activity of the preparations produced increases regularly, with small variation, according to the increase of starch. The other components of the chemical composition of bran that we studied – total nitrogen content, amino nitrogen content, and ash content – did not conspicuously affect the enzyme-producing activity of the mold, although the different brans had different quantities of these substances. In order to verify on a large scale the realtionship between the starch percent and the amylolytic activity we examined another 22 samples of wheat bran from various mills in Kharkov, L'vov and Rostov oblasts. The experimental data shown in Fig. 1 were gathered; this material is arranged entirely according to the relationship between the increase in amylolytic activity and the degree of increase of the percentage starch content of the brans.

If, as was done in the graph, the dotted lines delimit the zones not more than ± 10 amylase units on either side

TABLE 1. Chemical Composition of Different Samples of Bran and the Activity Produced from Laboratory Enzyme Preparations from Them (all data gathered in a dry-air environment, humidity 12-13%; A. oryzae strain No. 476-velvety)

Bran sample No.	Starch, %	Free N, %	Amino N, mg/100 g	Ash, %	SC of preparation
1	12.3	2.6	110.8	5.6	146
2	15 2	2.5	97.6	5.6	144
3	20.0	2.8	—	5.2	175
4	23.2	2.8	—	4.3	187
5	23.3	2.5	83,4	4.5	213
6	25.7	2.5	90.0	4.3	202
7	27 6	2.7	—	4.0	190

Note: The sign – indicates that a test was not made.

of the median line, and the experimental points lie within this zone (which is the range of deviation shown in two parallel biological tests), then, since the overwhelming number of experimental points are clustered in the zone, the relationship between the activity of the preparations and the starch content of the bran is graphically shown. It is true that a part of the experimental data seem to be on the limits of this provisory zone, but the majority of them are slightly outside the zone (3-5 amylase units), and only two of the 22 deviated more significantly.

Such a distribution of the experimental data is quite natural, since it could be expected that the activity of the preparations in all instances was correlated only with the starch content and that other components of the nutrient medium in the bran would show no effect. In all, in spite of the presence of the deviations, it may be regarded on a sound basis that the starch content, in percentage, in brans forms the major method of determining their usefulness as a nutrient medium for cultivating mold with the view of gathering amylolytic enzymes.

In all cases the use of thoroughly thrashed, thin wheat bran with starch content below 20% led to the production of enzyme preparations with strongly reduced amylolytic activity, and the use of such brans can not, therefore, be recommended.

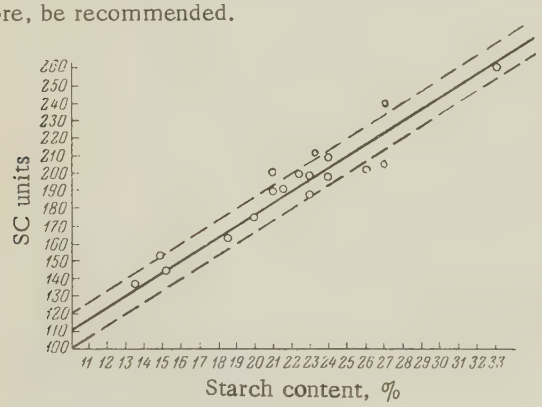


Fig. 1. The relationship between the percentage of starch content in the brans and the biochemical activity of the preparation.

TABLE 2. Amylolytic Activity of Preparations from Different Strains of the A. oryzae Mold in Comparison with the Bran Starch Content (in SC units)

A. oryzae strain	Bran starch content		
	15.2%	20.0%	29.7%
81	139	177	210
476-velvety	156	180	258
476-I	198	220	310

For verification, not only A. oryzae strain No. 476-velvety was used in these tests, but other strains used in production (No. 81 and No. 476-I) were produced by sifting, were placed in a nutrient medium prepared from bran with a different starch content, and the amylolytic activity of the preparations was determined (Table 2).

The results obtained were analogous. Thus, the relationship established is apparently general for the strains of A. oryzae studied.

If the starch content of brans is the predominant factor determining the amylolytic activity produced in their enzyme preparations, then the synthetic enriching of thin wheat bran by the addition of starch ought to increase the activity of preparations on such enriched brans. In a special series of tests two samples of thin wheat bran with starch contents 12.3 and 15.2% were taken and their starch contents raised to 22.3, 25.2 and 35.2% by adding potato and wheat starch and first grade wheat flour. Enzyme preparations with two strains of Asp. oryzae—No. 476-velvety and No. 476-I—were produced on such brans, and their amylolytic activity was determined. As a control several preparations not enriched by starch were used.

As can be seen from these data (Table 3), the addition of washed starch and wheat flour to non-starching brans does not influence the amylolytic activity of the preparations; the displacement in the relationship of starch to total nitrogen in the bran does not have any great significance.

On the basis of this it is necessary to come to the conclusion that the activity of the enzyme preparations is determined not only by the starch and total nitrogen in the bran, but also by other substances in the aleurone layer of the wheat grain lost in milling. Therefore the addition of washed wheat starch, potato starch, and wheat flour does not compensate for the deficiency in thin brans of the biologically important components and does not give the effect desired. The data produced indicate that the problem is far more complex. Nevertheless, for the practical goal of selecting raw bran for enzyme manufacture the starch content may be used as an indirect but sufficently reliable indicator. Of definite interest are the investigations we made on the effect of various wheats from which brans are produced on the manufacture of enzymes. With this view in mind eight samples of various sorts of wheat were obtained from the Kharkov plant: winter and spring wheat, hard and soft, red-grain and white-grain from the 1957 harvest. The groups were the following: winter, soft, red-grain—1) Lyutestsens No. 238, 2) Zenitka improved, 3) Ferrugineum No. 1239, 4) Lyutestsens No. 266; winter. soft, white-grain—5) Odessa, 6) Novo-Yur'ev; spring, soft, red-grain—7) Artemov; spring, hard, white-grain—8) Narodnaya.

Three samples of bran with different starch contents were obtained for each type from the milling plant. Enzyme preparations were obtained under laboratory conditions from strain 476-velvety of A. oryzae and their activity was determined. The results are shown in Fig. 2.

TABLE 3. Effect of the Addition of Starch to Thin Wheat Bran on the Amylolytic Activity of the Preparations

Mold strain	Bran sample No.	Total N in bran, %	Starch in original bran, %	Starch in enriched bran, %	Starch added	Starch: : total N ratio in bran	SC of preparation	Change in activity of preparation in comparison with control, SC units
476-velvety	1	2.6	12.3	—	—	4 : 1	127	—
	1	2.6	12.3	22.3	Wheat	9 : 1	122	— 5
	2	2.5	15.2	—	—	6 : 1	134	—
	2	2.5	15.2	25.2	Potato	10 : 1	123	—11
	2	2.5	15.2	25.2	Wheat	10 : 1	127	— 7
	2	2.5	15.2	35.2	Wheat flour, second grade	—	132	— 2
476-I	1	2.6	12.3	—	—	4 : 1	214	—
	1	2.6	12.3	22.3	Potato	9 : 1	224	+10
	1	2.6	12.3	22.3	Wheat	9 : 1	227	+13
	2	2.5	15.2	—	—	6 : 1	213	—
	2	2.5	15.2	25.2	Potato	10 : 1	220	+ 7
	2	2.5	15.2	25.2	Wheat	10 : 1	209	— 4

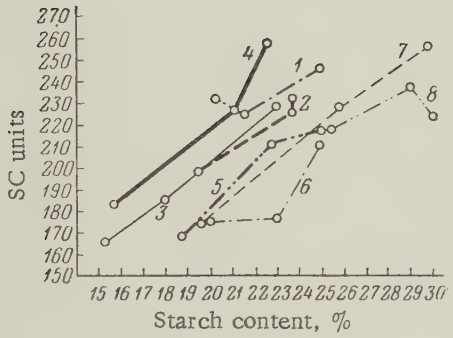


Fig. 2. The activity of enzyme preparations produced in bran from various types of wheat. 1) Lyutestsens No. 238; 2) Zenitka improved; 3) Ferrugineum No. 1239; 4) Lyutestsens No. 266; 5) Odessa; 6) Novo-Yur'ev; 7) Artemov; 8) Narodnaya.

If we examine the data obtained individually for each type of wheat, with the exception of two cases out of 24 the amylolytic activity in the enzyme preparations increased in proportion to the increase in the bran starch content. Along with this the variation between the individual types of wheat tested was made extremely apparent. For example, with the same starch content in bran samples from different wheat varieties (No. 6 – 22.8%, No. 3 – 23.0% and No. 4 – 22.6%) the activity of their enzyme preparations differed by 50 and 80 SC units. The most important brans for enzyme production came from wheat types Lyutestsens No. 266, Lyutestsens No. 238, Ferrugineum No. 1239 and Zenitka improved (in the figure they are correspondingly designated by No. 4, 1, 3 and 2). Because of the limited number of types tested we still do not have a basis for a final conclusion, but it should be noted that all these wheats came from a single group – winter, soft, red-grain wheat. The other varieties came from different wheat groups, and brans obtained from them were less favorable media for the growth of amylolytic enzymes.

The results would indicate that, along with the starch content of bran, other factors are significant, though they have not been precisely determined and though their components differ in the various types of wheat. Some attempts have been made by us to determine the nature of these substances which are significant to enzyme formation, but they have not given an answer to the problem posed. It might be surmised that they are related to the group of biologically active compounds of the vitamin type or irreplaceable amino acids. Nevertheless, the addition of extracts from malt seedlings and autolyzed yeasts, rich compounds of this type, and also of aqueous infusions of treated and nontreated enzymes from bran to thin wheat with washed starch did not lead to an increase in amylolytic activity in their enzyme preparations. Evidently these compound-forerunners, not necessary for the production of amylolytic enzymes, are high-molecular or are compounds contained in the cellular contents of the wheat grain which do not enter into aqueous infusion from these substances.

SUMMARY

1. Wheat bran is the most favorable nutrient medium for the formation of amylolytic enzymes in a culture of Aspergillus oryzae. Not all lots of wheat bran, however, are equally suitable for this purpose.

2. A known influence is exerted by the type of wheat from which the bran is obtained. Best results are obtained with bran from winter, soft, red-grain wheats.

3. Of greater importance is the starch content of the bran. As a source for the enzyme industry wheat bran from normally milled wheat with a starch content not less than 20-22% alone can be recommended. The use of highly milled wheat results in an abrupt decrease in the enzyme activity of the culture.

4. Fine milling causes starch loss in the bran as well as the loss of other unidentified compounds which are important for the formation of amylolytic enzymes.

LITERATURE CITED

1. E. Ya. Kalashnikov and D. B. Lifshits, Konsul't. po pishch. prom. (Kharkov, Izd. Ukr. NIIPP, 1946) 1 (36), 22.
2. A. Oparin and P. Yal'tsev, The Wheat Industry [in Russian](1929) p. 211.
3. R. V. Feniksova, F. M. Kinzburskaya and A. A. Shilova, Tr. Vses. n.-i. in-ta spirt. prom. 3, 110 (Pishchepromizdat, 1954).
4. T. Harada, Industr. Eng. Chem. 12, 1424 (1931).
5. K. Oschima and M. Church, Industr. Eng. Chem. 1, 67 (1923).
6. J. Takamine, Industr. Eng. Chem. 6, 824 (1914).
7. L. Underkofler, E. Fulmer and L. Schoene, Industr. Eng. Chem. 6, 734 (1939).
8. L. Wallerstein, Industr. Eng. Chem. 19, 1218 (1939).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. ***Some or all of this periodical literature may well be available in English translation.*** **A complete list of the cover-to-cover English translations appears at the back of this issue.**

MICROBIOLOGY IN CAIRO

A. A. Imshenetskii

Translated from Mikrobiologiya, Vol. 30, No. 3, pp. 545-549, May-June, 1961
Original article submitted February 24, 1961

There exists an understanding between the USSR and the UAR by which Soviet specialists go to the UAR to deliver lectures and give consultations. Consequently, by the agreement, I visited Cairo in the winter of 1961 and became acquainted with the work of local microbiological laboratories. Below some facts are given about the microbiological research being conducted in these laboratories.

SCIENTIFIC INSTITUTIONS IN CAIRO

The largest research institute of the UAR is the National Research Center, headed by Professor Turki, a leading specialist in the field of electrochemistry. The Research Center has a large number of well-equipped laboratories among which are: 1) solid state physics, 2) mineral raw materials, 3) chemical synthesis of medicinal compounds, 4) the synthesis of steroids, 5) paper manufacture, 6) textile manufacture, 7) ceramics, 8) cotton, 9) plant raw materials, 10) the milk industry, 11) biochemistry, 12) purification of sewage water, 13) chemistry of microbes, 14) agricultural microbiology.

Research in these laboratories has a predominantly applied nature and is conducted with inquiries on the national economy of the country. They perform a great service to medicine, industry, and agriculture. The scientific level of the leading work is rather high and of a completely up-to-date nature both in method and theory. Aside from the Center, research in microbiology is carried out at the University of Cairo in two faculties; by the science faculty, in which the chair of microbiology is held by Mustafa Abd-al-Aziz, and the agriculture faculty, in which the chair of microbiology is held by Prof. Abd-al-Malik. In the other university in Cairo, called Ain Shams University, there also exists a chair of agricultural microbiology, headed by Professor Taha. In addition to this, microbiological research is conducted in the laboratory of agricultural microbiology under the supervision of the Ministry of Agriculture and the "Naser" Company, which is starting to build an antibiotics plant by plans developed in the USSR.

GENERAL TREND OF WORK IN THE FIELD OF MICROBIOLOGY

The above-listed science-research institutes conduct research primarily in the field of technical or soil microbiology. There are no laboratories of general microbiology in the country, and such divisions of microbiology as taxonomy, microbes, theoretical physiology and biochemistry, ecology, and others generally do not exist. Apparently the problems of industry and agriculture prevail and determine the trends of microbiology. This undoubtedly has a great advantage for conducting research, since the results are practical and in many ways assist the effective development of the country's economy. It is also impossible not to note that such limitations in science do away with the possibility of discussion of further theoretical and practical development. It is necessary to point out that research in the field of microbiology is, on the one hand, combined with problems of soil productivity and plant cultivation, and, on the other hand, has an immediate relationship with the chemical, food and pharmaceutical industries.

POSITION OF MICROBIOLOGY ON SPECIFIC PROBLEMS

Physiology and Biochemistry of Microorganisms

Considerable interest is shown in research on the physiology of molds. In the Research Center the laboratory of microbe chemistry chief Dr. Taha is studying the effect of microelements on Aspergillus niger and its capacity to produce citric acid. He determined that Zn is important in the biosynthesis of citric acid, while Mn and Cu do not show such action. Moreover, the addition of Mn to a culture depresses the activity of the mold. The production of citric acid is stimulated by methyl and ethyl alcohol, which also do away with the adverse effect of Mn. Research on the enzyme systems of some molds participating in the decomposition of uric acid and purine bases was also done with this research. A purified uricase preparation produced has adequate activity. At the University of Cairo Professor Hussain Said, in a chair of microbiology, is conducting research on the permeability of sucrose through the cell wall of Fusarium mycelium. Sucrose penetrates the mycelium of a mold only after its hydrolysis. The enzyme action is hydrolytic in nature, and phosphorylase action is negated.

In the biochemical laboratory of the Research Center the biosynthesis of vitamin B_2 (riboflavin) in cultures of Eremothecium eschbyii and Ashbya gossypii is being studied. The optimum pH and rH values for the synthesis of this vitamin in a mold culture have been established. Conditions of vitamin B_{12} production by microorganisms are also studied in this laboratory.

Both in the Research Center and in the chairs of microbiology at the universities experimental research on the biological fixation of nitrogen is conducted. Attention is focused on the nitrogen-fixing capacity of blue-green algae and on clarification of the most favorable conditions for maximum biochemical activity in algae.

The problem of protein is of more than theoretical significance to Egyptian scientists. Vegetable proteins are the only protein available to the bulk of the population, and the tendency of microbiologists to assist nutrition physiologists in finding a solution to this problem is, therefore, understandable. The synthesis of proteins by algae and molds is intensively studied, and the possibility of isolating plant proteins from wild plants growing along the banks of the Nile is being investigated. In this type of work it is necessary to focus research on the fat accumulation of molds; some species of Aspergillus produce up to 40-50% fat by dry weight of the mycelium.

Technical Microbiology. In the study of acetone-butanol fermentation, bacteria are cultured in a partially defined medium, which makes it possible to examine the different amino acid and vitamin requirements of acetone-butanol bacteria. Considering the possibility of culture contamination by the production of lactobacilli, a search for antibiotics which will depress the activity of extraneous bacteria and not influence the stimulation of acetone-butanol fermentation is being carried out. In Egypt all sugar is made from cane, and there is, consequently, much molasses in the country. It is used partly for alcohol production, but significant amounts are not used in Egypt but are exported. Microbiologists and biochemists are, therefore, working intensively on the technology of producing various substances from molasses with the help of microorganisms. The problem of mannitol and glycerol production by such a method was successfully solved, as was production of citric acid by the surface method. Research is also conducted on the utilization of hydrolysis products from corn stalks for the growth of pentose yeasts, used in cattle fodder. Microbiologists give considerable attention to the maceration of such fibers as flax and local hemp. This work is carried out both at Ain Sams University and in the laboratory of microbiology of the Ministry of Agriculture. Hemp maceration is done in special, long, concrete tanks with flowing water. The method is completely original and, to some extent, suggests a continuous microbiological process.

After removal of the external, thicker part of the stalk by a mechanical method, the hemp sheaves are placed in a terminal tank, after which they are moved forward for a day in a current of fluid. The movement of sheaves and the introduction of new sheaves in the free space is done 15 times in 15 days that is, this is the time for complete maceration. Each day a lot of macerated sheaves is removed from the "starting" tanks and a new lot is placed in them. In this way the bacteria that ferment pectin as a fermentation product are not depressed and the problem of adding fluid is done away with completely. In microbiological analysis both Clostridium felsineum and Granulobacter pectinovorum are used. The study of the microbiological processes in the fermentation of vegetables was done in three phases, for each of which particular microflora are characteristic. At first nonspecific microflora consisting of sporogenous species of Aerobacter, Leuconostoc and some species of heteroenzymic lactobacilli are developed. It proceeds with the formation of homoenzymic lactobacilli of the Lactobacillus plantarum type, accompanying the formation of lactic acid. In the third phase the development of heteroenzymic lactobacilli, of which Lactobacillus brevis and similar yeasts are typical, takes place.

The fact that a persistent search in the soils of Egypt for Bacillus botulinus has not been successful is of definite interest to the canning industry. Thus, local canning can not be effected by the toxic effect of B. botulinus. In the Research Center's laboratory for the synthesis of steroid hormones a study of hormone transformation by means of microorganisms has been started.

Research on the experimental variability and selection of microbes is practically nonexistent in Egypt. The exception is work on the adaptation of yeasts to an increased concentration of sugar or alcohol in their environment. Yeasts that reproduce sexually become acclimatized and, furthermore, are assumed to cross with osmotolerant and acid-yeast strains.

Microbiology of Milk and Milk Products. The milk industry of Egypt has a completely unique nature. Buffalo milk, which contains considerably more fat than cow milk, is used for the manufacture of a number of national milk products. Microbiologists have studied the microbiological processes coming from the preparation of lactic acid products and cheeses, the surfaces of some of which are covered by yeast consisting of protein proteolysis-producing bacteria. The variation in the activity of renin (chymosin) used in cheese manufacturing for the production of curd prompted Dr. Taha to point out the possibility of substituting for renin preparations of proteolytic enzymes produced from cultures of various molds and having an activity at pH 4.5-5.0. Such preparations were produced from cultures of Fusarium, Penicillium and Aspergillus and were used with success instead of renin. A lactic acid product completely unique to Egypt, "kishk," produced microbiologically, was studied in the agricultural faculty of Cairo University. Skimmed buffalo milk is slanted and casein curd is consequently produced. At the same time wheat grain is soaked and afterward placed in a fine layer in a warm place. Bacillus subtilis reproduces on the surface of the grain, hydrolyzing out the amylase starches of the grain. Then the casein curd and wheat grain are mixed and are then allowed to ripen for some time, after which a paste is formed by the action of the lactobacilli and the yeast. The mixture is formed into balls which are dried. Such a concentrate, containing protein, carbohydrates, vitamins

and organic acids, is easily transported and is used by the people in place of butter and eggs in the preparation of various foods.

Soil Microbiology. Egypt is a country of unusually intensive farming. The fertility of the Nile valley is well known, but a number of unique soil characteristics present some problems to the microbiologist. It is natural that a three-crop-a-year harvest presents an important fertilizer problem. Along with the use of significant amounts of mineral fertilizer, green fertilizer—that is, plowed-under green plants, mainly clover—is widely used. The decomposition of organic matter in the soil has been successfully studied by Professor Malik both at the Research Center and in the microbiology chair of the agriculture faculty at Cairo University. Plant refuse is broken down quickly due to irrigation, a sufficiently high temperature and the alkaline reactions of the soil. In the long run the relationship between carbon and dissolved nitrogen in the soil is displaced and equals 2 : 1 or even 1 : 1. Nevertheless, the character of these nitrogenous compounds is still not established. At the same time the microbiological and biochemical processes taking place in the production of various composts are being studied. The appreciable warming of the soil in summer produces seasonal variations in the number of microorganisms contained at different times of the year. With regard to this, each month at Ain Shams University the chair of microbiology determines the total number of microbes in the soil by cultivation in a dish with a thick medium, and also the number of sporogenous forms, Azotobacter, nitrogen-fixing Clostridia and nitrifiers by the dilution method. Both the total number of microorganisms and of representatives of specific groups increases in May, after which, in conjunction with the warming of the soil, it decreases and then increases again in autumn. In this way the curve corresponding to the number of microbes is double-apexed. The chair of microbiology also investigates microbiological methods for determining the potash and phosphorus requirements of the soil. A. niger and Azotobacter are used as organism indicators. The ability of specific strains of nodule-forming bacteria to form nodules is also studied by means of sterile lentil cultures. In the laboratory of microbiology of the Ministry of Agriculture research is conducted with regard to the pH variation of soil being surveyed after the introduction of sulfur and its oxidation by bacteria. This is of considerable importance, since the pH of many Egyptian soils is 8.0-9.0. The head of the chair of microbiology in the science faculty at Cairo University, Mustafa Abd-al-Aziz, pays considerable attention to phytopathology and, in particular, to the infectious diseases of cotton. The ecology of inducers and methods of prevention, among which particular interest is offered by the introduction of antagonistic Trichoderma, is studied. In field conditions this method gives good results with simultaneous soil acidification, for Trichoderma reproduces poorly in alkaline soils.

Geological Microbiology. The activity of bacteria taking part in the sulfur cycle has considerable importance for Egyptian economy. The subsoil is enriched by sulfates from the water of the Nile, and the sulfate reduction leads to the formation of sulfides. Furthermore, carbonates are produced and the pH of the soil is significantly increased, which is undesirable. An analogous process is the formation of carbonates in lakes. Forty km from the Nile is an alkaline lake, surrounded by marshes in which sulfate reduction, owing to the presence of sulfates and organic matter, is seen. The number of desulfurizing bacteria sharply increases, the sulfate content drops, the pH reaches 8.5, and as a result of the formation of bicarbonates and carbonates the water, entering the lake from the marshes, contains the latter in such large quantities that in summer time, owing to the partial evaporation of the lake water, precipitation of soda, used in the production of soap and for other requirements of chemical manufacturing, is produced. From the scientific viewpoint it is interesting to note that the quantity of carbonates produced is greater than may be theoretically explained from the amount of sulfates in the water.

Personnel. The training of microbiology students in the universities is accomplished on a sufficiently high theoretical level. For the doctor of science degree young scholars go to the universities of England, the USA, or the Federal Republic of Germany. In the past year more and more university graduates tend to go to the USSR. In the head chairs are prominent, scholarly scientists, who are creating an independent direction in research work. The staff of chairs and research laboratories as is natural, consists of men solely. Among approximately 50 microbiologists in Cairo three are women: a specialist in antibiotics, a virologist, and a fellow in the laboratory of milk microbiology. Microbiologists directing the laboratories in the Research Center are undoubtedly well-trained, very capable, young scientists from 30-35 years old, speaking two or three western languages. Both laboratory directors and their staff workers have excellent training in physics and chemistry. Therefore, the level of research in the fields of microbial chemistry and technical microbiology is very high. Least attention is given such problems as the structure, taxonomy, ecology, evolution and variability of microorganisms. The Egyptian microbiologists select the most interesting and important of the practical scientific details of other countries for their research methods and theory. Nevertheless, such borrowing has not hindered the creative development of a variety of problems of interest to the country's economy.

ECOLOGY OF Nitrosomonas

O. M. Ul'yanova

Translated from Mikrobiologiya, Vol. 30, No. 3, pp. 550-564, May-June, 1961

In the course of the past 40 years the formation of a new branch of microbiology has taken place—the ecology of microorganisms. An ecological approach to the study of the functional and morphological characteristics of microbes is all-important in microbiology. Investigators have given considerable significance to the natural habitat of microorganisms, emphasizing the necessity of detailed analysis of the metabolism of microorganisms and the ecological surroundings to which they are adapted [Winogradsky, 1952; Seliber, 1955, 1960; Isachenko, 1938; Mishustin, 1947; Imshenetskii, 1944, 1952, 1956; Lazarev, 1949; Krasil'nikov, 1958; Kuznetsov, 1955; Suskhina, 1952; Rubenchik, 1959; Loginova, 1960; Lyalikova, 1959; Garrett, 1955; Rehm, 1954; Szabo and Marton, 1958; Thornton, 1956 and others].

THE ADJUSTMENT OF NITROSOMONAS TO ITS CONDITIONS OF EXISTENCE

In the works of numerous authors it is shown that nitrifying bacteria and in particular stimulants to phase I of the nitrification of Nitrosomonas are prevalent in various natural substrata. They live in the soils of various countries and parts of the world (Europe, Asia, America, Africa, Australia) including the Arctic and Antarctic, in the water of seas and in fresh water, in mud, in estuaries, on the surface of limestone, granite, brick buildings, in decomposed and fresh rubbish, in manure, and in many other natural environments.

At present there is a great amount of material on the physiological and biochemical characteristics of nitrifying bacteria which has been brought together in a series of summaries[Lees, 1954; Meiklejohn, 1954; Ruban, 1956; Novikova, 1960]. The connection between the environment of Nitrosomonas and its morphological and physiological characteristics has, however, still not been very thoroughly studied. Often works concerning research on nitrifying bacteria fail to indicate the environment from which the cultures came, and their chemical characteristics are not referred to. In the majority of works the investigators define only the presence of nitrites and nitrates in them or their natural substrata; sometimes the number of bacteria is given but then only isolated in a pure culture. It is, therefore, impossible to draw any conclusions concerning the ecology of Nitrosomonas on the basis of such work. Only isolated works concern themselves with this important aspect in the study of nitrifying bacteria. In going over these works our attention was caught by one section of a summary. The basic ecological environments of these bacteria are different in soils in which the oxidation process of ammonia to nitrites and nitrates goes on at the expense of bacterial activity [Winogradsky, 1952; Waksman and Madhok, 1937; Meiklejohn, 1953, 1954]. We will examine the peculiarities of the nitrification process in these environments.

RATE OF NITRIFICATION IN VARIOUS TYPES OF SOIL

The nitrification process in soils depends not only on the presence or absence in them of nitrifying bacteria but also on a number of other factors. The genetic soil type, its treatment, the plant cover, the moisture in the soil, the relationship between its carbon and nitrogen, the climatic conditions of that or another region—all exert considerable influence on the course of the process. A substantial difference in the percentage of organic matter in the soil, its degree of acidity, the concentration of oxygen in the soil, the ground reserves which affect the physical characteristics of the soil, the rapidity of the depletion of the nitrogen reserves in the soil or the scarcity of easily utilized nitrogen—these also emphasize the contrast of conditions in the environment of microorganisms. In the works of both native and foreign investigators problems are discussed in connection with the rate of the nitrification process in various soil types. The basic conclusion of these researchers may be boiled down to the fact that vigorous nitrification is observed only in soils where there is intensive decomposition of organic matter and where there are significant quantities of ammonium producers [Kostychev, 1930; Korsakova, 1930; Yashnova, 1930; Tyagny-Ryadno, 1933; Smalii, 1950; Kolcheva, 1954]. A correlation between the spread of some species of spore-forming ammonifiers in soils (Bacillus idosus, Bacillus mesentericus, Bacillus megaterium, Bacillus mycoides) and the increase in these soils of the nitrification process has even been noted [Mishustin and Pertsovskaya, 1954; Pushkinskaya, 1954; Mitrofanova, 1953]. In such substrata it is evidently normal for nitrifying bacteria with a high oxidizing capacity to live. In environments where the biochemical processes move more slowly strains of nitrifying bacteria with insignificant nitrifying capacity grow. We may, therefore, speak here of definite ecological types of nitrifying bacteria adapted to life in specific natural substrata. This situation is confirmed by the

data gathered through a study of the nitrification process in various soil types.

The soils of the far north (tundra, sandy loam, sandy, and others) are characterized by high acidity (pH 3.5-3.7), unusual paucity of organic matter (1.9-2% and less) and by a weak manifestation of the ammonification process [Tsyplenkii and Zhilin, 1936]. In these soils weak nitrification is observed and here the process is often entirely absent [Severin, 1909; Burgvits, 1930; Kazanskii, 1932; Isachenko and Simakova, 1934; Levinskaya and Mamicheva, 1936; Kriss, 1947; Mishustin and Pertsovskaya, 1954; Zhukova, 1956]. It is clear that the nitrification process is developed only in cultivated soils, especially in manure fertilizer. With a change in the ecological condition of these soils—an increase in alkalinity, an enrichment in organic matter and mineral fertilizer, a change and regulation of the thermal system of the soil, etc.—a more favorable condition for the development of Nitrosomonas is created. The too-acid reaction of high-altitude soils (varying in pH from 4.1 to 5.5), the small number of ammonifiers in them, and the moist, cold climate of high mountain regions limit the development of nitrifying bacteria in them. According to Lopatina [1930], the potential nitrification capacity of the mountain-meadow soils of the south Caucasus is very low. In the mountain-meadow soil of the Caucasus range, at a height of 2,200 m, in the mountain soils of south Ossetia and the mountain soils of Bulgaria, at an altitude of 1,700 m above sea level, the nitrification process and nitrifying bacteria are absent [Simakova, 1932; Domracheva, 1933; Kolcheva, 1954; Chodzički, 1954].

Authors have noted the presence of nitrification in various desert soils, but the process occurs weakly in them. In limited numbers nitrifiers are found in the sands of Turkestan [Shul'gina, Beresneva, Norkina, 1930]. Feher [1946] found them in the sandy soils of the Sahara Desert in quantities from 100 to 10,000 in 1 g. Kostychev and Kholkin [1930] noticed the nitrification process in the Bukhara nitrite deposits (an accumulation of nitrites in hills of desert clay). Noticeable nitrification was observed only in flat desert locations in some dune sections where salting was slight. Pontovich [1950], in a study of samples of various soils from the high-altitude Pamir deserts, discovered nitrification and active nitrifying bacteria in 99 of 118 samples tested, amounting to 74.5%. Nitrifiers isolated from soils of the eastern Pamirs were better developed when crushed granite, the predominating rock in that region, was added to the thin mineral environment. In an environment with granite a positive nitrite reaction appeared on the 16th day, but in the normal Winogradsky medium—only on the 46th day. The Pamir Nitrosomonas also have a considerable need for iron salts.

Typical red-earth soils (the Batum coast) are characterized by weak nitrification [Garder, 1929; Shvetsova, 1931; Obraztsova, 1936]. This depends on a series of factors limiting the growth of Nitrosomonas: considerable lack of soil saturation by bases, insignificant Ca cation content, an acid soil reaction (pH around 4-4.6), etc. Even the mineralization of organic nitrogen and ammonia, very slightly used by nitrifying bacteria, takes place slowly in them.

In natural podzol soils the lack of easily moveable nitrogen and an acid reaction limits the nitrification process. Nitrifying bacteria are often quite limited in podzol soils. The alluvial level of turf-podzol soils does not contain nitrifiers [Fedorov and Smirnova, 1953]. Investigators do not note a substantial difference in the nitrification energy of podzol soils in different climatic zones [Burgvits, 1928; Sheloumova and Berg, 1928; Shvetsova, 1930; Korsakova, 1930; Bylinkina, 1928; Lopatina, 1930; Nikitina, 1930]. Cultivated podzol soils have significant nitrification activity. So, for example, maximal nitrifying capacity and the greatest number of nitrifying bacteria were characteristic only of tilled turf-podzol soils with a high agricultural base [Nizova, 1960; Avdonin, Arens, Stepanova, 1960].

Sandy soils, according to the research of a number of authors, also have a low nitrifying capacity [Remezov, 1951; Glotova, 1950; Gerretson and de Hoop, 1957; Sakai, 1959]. According to Remezov's data the sandy soils of the river Desna terraces, located on alluvial sands with pine forests having coarse humus (1-2%), an acid reaction (pH 4.5-5.5) and a low base saturation (10-20%) have a weak manifestation or absence of the nitrification process. sandy soils of pine forests having 3-4% humus, a high base saturation (70-80%) and a weak acid reaction (pH 5.5-6.5) have more significant nitrification. In the sandy soils of Hungary significant numbers of nitrifiers—1,000 per g of soil—were found in a deep deposit of manure and compost in them [Varga and Dyurko 1955]. Chandra and Bollen [1960] controlled an increase in the nitrification process greater than 100% in the sandy-clay soils of Oregon by adding small doses of potassium salts and gibberellic acids. The presence of nitrifiers and a weak nitrification process in ordinary sandy cultures of pea plants was discovered by Shulov [1911].

Natural peaty soils, in spite of their wealth of organic matter and nitrogen, are ecologically unfavorable for Nitrosomonas. The nitrification capacity is very low or absent. The inertness of natural peat soils is noted by investigators both for the different soil-climatic regions of the Soviet Union [Korsakova, 1930; Kholkin, 1930; Burgvits, 1928; Kostychev, 1930; Sushkina, 1932; Levinskaya and Mamicheva, 1936; Pertseva, 1950; Garkusha, 1953; Vavulo, 1955; Kozlov, 1956] and for those of other countries. Golebiowska and Falkowski [1953] note this for the peats of Poland; Boswell and Sheldon [1951] – for English peats; Stevenson [1953] – for the peats of the Marshall Islands; Coppier and de Barjac [1952] – for the peats of France; Turk [1939] – for the peats of Michigan. The main role for the increase of peat soil biological activity is played by cultivation. According to Korsakova, cultivated peat

soils are different from all soils where, under natural conditions, nitrifiers produce enough material for the accomplishment of the nitrification process, since the nitrogen stock of peat soils is very great. In the arable layer of peat soils, up to 28-29 mg and more of nitrates for each 100 g of soil are accumulated [Garkusha, 1953]. It is interesting to note the nature of nitrifier growth and the accumulation of nitrites in peat composts used for fertilizing fields. According to Kiseleva's research [1958], the nitrification process in peat composts depends not only on the activity of the nitrifying bacteria themselves but also on microorganisms using nitrates, primarily aerobic cell tissue destroyers and, in part, denitrifiers. The author notes the specific action of these three groups of microorganisms on one another. According to the data of Berezova and Rempe [1958], the increase of the nitrification process in peat composts is controlled by Azotobacter, Clostridium pasteurianum and denitrifiers.

Humus-carbonate soils have a high nitrifying activity. This is characteristic both for humus-carbonate soils of the north [Garder, 1930] and for humus-carbonate soils of the south [Sheloumova and Berg, 1928]. High nitrification intensity promotes a favorable combination of physico-chemical and mechanical characteristics for soils of a given type: a weak alkaline reaction, large amounts of calcium, and base saturation. Here the limiting factor for the nitrification process is the small amount of organic nitrogen and the insignificant rate of its mineralization.

Chernozem soils have the greatest nitrification activity. The most characteristic trait of these soils is the lack of difference in microbiological activity between cultivated and natural soils. This has been stressed by a number of investigators working with a variety of chernozems–ordinary, thick, leached, mountain, etc. [Korsakova, 1930; Shvetsova, 1930; Burgvits, 1928; Kardo-Sysoeva, 1928; Lopatina, 1928; Beresneva and Shvetsova, 1928; Prostakov and Mina, 1945; Runov, 1954]. More peculiarity is shown by Kolcheva [1954] for the bituminous chernozems of Bulgaria. The accumulation of nitrates in chernozem soils is limited only by the nitrification material. The number of microorganisms themselves in chernozems at all times of year is quite sufficient for the oxidation of a considerably larger amount of ammonia than can be produced in the soil. The activity of chernozem may be increased by strengthening the ammonification in it and also by the introduction of mineral fertilizers. The introduction of manure and phosphates is favorable only for eroded and malted chernozems [Tyagny-Ryadno, 1933; Taranovskaya and Germanov, 1927]. According to Lopatina's research mountain chernozems and lowland chernozems have high nitrification activity. Large amounts of nitrates form leached chernozems in the foot hill gravels of southern Ossetia [Prostakov and Mina, 1945]. In 1 kg of dry soil in the arable layer of fallow chernozem there are accumulated up to 25-30 mg of nitrogen as NO_3 in January. Here the intensive nitrification is combined with considerable intensity in the ammonification process.

Cultivated sierozem under irrigation has a very high potential nitrification activity [Burgvits, 1928; Sheloumova and Berg, 1931; Kononova, 1930]. Here the rate of mineralization of nitrogen outstrips the rate of nitrification significantly. For these soils irrigation without the use of fertilizer is all that is necessary. Sierozems are significantly more active in nitrifying capacity than chernozems [Sheloumova and Berg, 1931]; this has been noted, for example, for the fertile sierozems of Kaunchi (northern Asia).

In solonetz soils the nitrification processes is very intensive. A number of investigators [Butylin, 1936; Danini and Kosmortov, 1933; Germanov, 1933; Keller and Karel'skaya, 1926] brought to light the fact that the nitrification process in solonetz soils is more intensive than in chernozems.

In solonchaks, according to the research of Genkel' and Danini [1936] and Genkel' and Butilina [1935], nitrification is absent. In an analysis of the solonchak of Troitskii district it was the opinion of Genkel' and Danini that it had unfavorable conditions for nitrifiers: a high osmotic pressure in the solonchak solution (13 atm), a salt quality (Glauber salt acts more negatively than table salt), a negligible production of organic matter and, as a consequence of this, no certainty for nitrifiers of ammonium nitrate. According to the data of Kovda and Selyakova [1935], the solonchaks of northern Asia contain a vast amount of potassium nitrate.

Panosian [1948] notes that the nitrification energy of the Armenian solonchaks has a high concentration of chlorides, sulfates and carbonates. The largest number of nitrifiers was found by the authors in eroded solonchak. In eroded solonchaks up to 10.37 million nitrifying bacteria were counted in the surface strata, in "fluffy" –8.9 million, and in moist–up to 6.33 million per 1 g of soil. In the course of 25 days up to 12.86 mg nitrogen was oxidized to HNO_3 from 0.5 g $(NH_4)_2SO_4$ in eroded solonchak, and in moist solonchak only up to 7.34 mg.

The nitrifiers from these soils grew better in media made from solonchak extracts than in a synthetic Winogradsky medium. Growing for a long time under conditions of high salting, nitrifying bacteria adapted themselves to the high osmotic pressure of the soil solution.

All the material tests indicated that there is no doubt concerning the adaption of nitrifying bacteria to the ecological nature of various soils. It is necessary to know this in order to plan favorable laboratory conditions for them.

THE EFFECT OF VARIOUS PLANT CHARACTERISTICS ON THE NITRIFICATION PROCESS IN SOILS

Plant charachteristics also show a definite effect on the nitrification process in soils.

In various forest soils (soils of pine and spruce forests with a cover of grass, bryophyta., heather, lichen,

foxberry, blueberry), the nitrification process proceeds very weakly or does not take place at all [Sushkina, 1933; Mal'chevskaya, 1933; Shtobe, 1952; Kolcheva, 1954; Chase and Baker, 1954]. Shumakov [1948] has explained the reason for the depression in the nitrification process in forest soils by the peculiarities of the organic matter. Different water-soluble products of fall shedding and the forest flooring produce a depressing effect on nitrifier growth, according to this data, analagous to the action of rosin, tannin and bitumen.

The stimulating of the nitrification process by cutting forests is noted in a series of works [Remezov, 1941; Sukhareva, 1957; Sushkina, 1931]. Ofanitskii, Fedchenko and Ardashev [1960] report on the favorable effect of burning and subsequent planting with rosebay cuttings (Epilobium angustifolium on the nitrification process (Arkhangel oblast). The soil acidity changes (from pH 5.3-5.8 to pH 6.1-6.7), the nutrient system and aeration improve, and there is a temperature increase of 2-3C in the upper soil horizons. The number of nitrifying bacteria in the soils of Epilobium cuttings is 100 times greater than that in regular forest soils [Tvorogova, 1959].

It is indicated in a number of works that the conditions in soils used for forest plantings are favorable for the growth of nitrifying bacteria. Thus in a low-humus, carbonate chernozem under plantings of yellow acacia and maple, on the steppes of Kazakhstan, the number of nitrifying bacteria, according to the observations of Mitrofanova [1953], was higher than in the virgin steppe soils not used for forest plantings. Forest plantings enriched the soil in humus, in the elements of mineral nutrition, and formed the best hydrothermic system for an increase in the activity of the process. The nitrification process is intense in soils used for cultivation and in garden soils. Mal'chevskaya [1938] discovered the same high nitrification in soil with a cover of fodder grass, only it was weaker under oats. In meadow-irrigated soils in a zone of the middle Amu-Darya under cotton and alfalfa cultivation nitrifying bacteria were found by Kiseleva [1955] and in a group of young cotton plants in salted soil by Naumova [1954]. A large number of nitrifiers were found by Stevenson and Chase [1953] in garden soil, especially if it were covered by not completely decomposed straw and alfalfa. In greenhouse soil the presence of nitrifying bacteria was noted by Klein [1955]; in it the growth of a culture of Nitrosomonas was significantly accelerated by substituting a Winogradsky medium of ammonium sulfate and ammonium borate.

The rhizosphere of some trees, in particular the oak [Manteifel', Zhukova, Dem'yanova, 1950] and the ash, is favorable for the nitrification of bacteria; under the canopy of the ash the maximal nitrification intensity in dark-gray forest-steppe soil was 53.5% higher than under the shade of oaks of the late and woody varieties [Shumakov, 1960].

According to Dorosinskii's data [1954], nitrifiers occurred in a sufficiently significant quantity (0.1 thousand to 1 g turf podzol soil and 20 thousand in 1 g humus-carbonate soil) in the roots of oats planted in turf-podzol and humus-carbonate soils. Evidently microflora growing in the immediate proximity of the roots and on the roots themselves and mineralizing organic matter create the necessary conditions for their activity. Novogrudskii [1959] noted the greatest number of nitrifying bacteria in the root system zone of beets.

Voroshilova [1954], studying the microflora of the rhizosphere of kok-saghyz, also found a larger number of nitrifiers in the root-system soil than in the soil between rows. They were found by Kriss [1947] in the rhizosphere of various plants of the tundra and polar desert soils of the Arctic.

THE ADJUSTMENT OF NITROSOMONAS TO THE ENVIRONMENT

There is very little material in the literature directly devoted to a study of the ecology of Nitrosomonas. Nevertheless, knowledge of them shows that the adjustment of nitrifying bacteria to the conditions of their environment may be diverse.

THE RELATIONSHIP OF THE MORPHOLOGY OF NITRIFYING BACTERIA TO THE ENVIRONMENT

Winogradsky [1952], who isolated Nitrosomonas from soil samples from Europe, Asia, America, and Austrialia, noted a number of peculiarities in the morphology and activity of various species of nitrifying bacteria. Vigorous nitrification was observed by Winogradsky in the soil samples from South America (Ecuador), in which the full completion of the process took one week. In the soil samples from other countries the process was completed in three weeks or more—in 40 days. Some of the soil samples generated the growth of monadiform nitrifying bacteria—others in zoogloea form (liquid media). In a dense medium dark and light-colored colonies developed. The components of the dark colonies in form and structure did not differ from the zoogloea of the liquid medium; the light ones consisted of monadiforms. In the soil samples from Africa, Winogradsky found characteristic zoogloea forms of nitrifying bacteria. In these samples nitrification took place very sluggishly—oxidation of various amounts of ammonia was 2-3 times slower than in European samples. Large cocci (1.5-2 μ in diameter) were found in the American and Australian samples; the zoogloea form was not found. The zoogloea nature of nitrifier growth from the soils of North Africa, as determined by Winogradsky, may be the result of adaptation to the dry climate of this region. Nitrifying bacteria isolated from the soils of different countries varied in their morphological characteristics and were kept separate by individual species. The Western European species Nitrosomonas europae, isolated by Winogradsky from the soils of Zurich and from irrigated fields near Paris, have oval cells 1.2-1.8 μ long and 0.9-1.0 μ wide, moved by a single short flagellium.

The species of Nitrosomonas in the soils of Eastern Europe (Kazan) have double cells smaller in size. The species of Nitrosomonas isolated from Asian soils differ from the European species in smaller cell size (soil sample from Tokyo, Japan). Even smaller in size is the Nitrosomonas species from the island of Java—Nitrosomonas javanensis (0.5 × 0.6 μ)—with a very long flagellum exceeding the cell diameter by 60 times. In addition the morphology of Nitrosomonas europaea has been studied by many investigators [Boltjes, 1935; Imshenetskii, 1945; Lees, 1953; Meiklejohn, 1950]. Last year a detailed study of the morphology of Nitrosomonas was made by Rudan [1960], who showed that the strains of Nitrosomonas studied are lophotrichous and that the growth cycle of Nitrosomonas, through its study with anoptral, electron, and phase-contrast microscopes, agrees with Winogradsky's data but may be broken down into four stages instead of the two detected by Winogradsky: 1) the fastening of cells to sediment or to the vessel base and their immobilization (3rd-4th days); 2) the majority of the cells have flagella, are actively moving, almost no fastened cells (5th-8th days); 3) the cells are in the dividing stage, some shed their flagella (8th-10th days); 4) the cells are without flagella, they produce zoogloea-like masses in the sediment (10th-12th days). Onset of motility usually coincides with a more intensive accumulation of nitrites. The transition to the zoogloea stage occurs at the time of the disappearance of all ammonia from the environment. According to the data of Lozinov and Ermachenko [1960], in the strains of Nitrosomonas europaea studied by them free cells and zoogloea forms differ not only in morphological characteristics but also in pigmentation. The zoogloea form is almost colorless or has a light brownish color. The free cells contain a red-brown pigment which has a hematite nature. Both forms actively nitrify $(NH_4)_2SO_4$.

Many authors have noted that Nitrosomonas europaea endures drying well and may be kept viable in dry oil samples for a long period—from three to 10-20 years [Fraps and Sterges, 1932; Meiklejohn, 1954; Imshenetskii, 1955; Calder, 1957; Ruban and Ermachenko, 1958]. It may also be exposed to lyophilic drying without a protective colloid substrate and is kept well in empty ampules for five years. Nitrosomonas cells are stable toward the effect of x-rays, not losing their viability with doses of 50 kr. Nitrosomonas endures unfavorable conditions better in the zoogloea stage than in the mobile cell stage [Ruban, 1960]. The nitrifying bacteria of the New World (Australia) have species of large immobile cocci 1.5-2 μ in diameter. Winogradsky combined them with the genus Nitrosococcus, concerning which there is very scant information in the literature. Mason-Williams [1959] found Nitrosococcus europaea in cave earth from Great Britain. Determination of the adjustment of nitrifying bacteria and of their physiological characteristics were made.

ADJUSTMENT OF NITROSOMONAS TO THE HYDROGEN ION CONCENTRATION IN ITS ENVIRONMENT

The maximum concentration of hydrogen ions for the growth of Nitrosomonas in a mineral environment, though it has not been precisely determined, is generally alkaline. This is demonstrated by the fact that various investigators have dealt with different strains of nitrifying bacteria which were isolated from different ecological conditions in the environment and were adapted to the determined reaction of the environment. According to Meyerhoff [1916, 1917], Hofman and Lees [1952, 1953], Nelson [1931], Bömeke [1939], da Silva [1952], Boswell et al. [1954], and Engel and Alexander [1958], the maximum pH for oxidation of ammonia by Nitrosomonas lies in the alkaline range (pH from 6.8-7.5 to 8.5-9). Gaarder and Hagem [1922] noted that there are some strains of nitrifying bacteria isolated from the 140 samples of different soils with various ranges of optimal acidity: α(7.7-7.9), β(6.7-6.8) and γ(6.5). Winogradsky and Winogradsky [1933] found a wider range of favorable acidity for various species of Nitrosomonas: Nitrosomonas a and d—7.4-7.8; Nitrosomonas b—8.6-9.2; Nitrosomonas c—7.4-8.8; Nitrosomonas e—8.6-8.8. Hofman and Lees [1953] pointed out that Nitrosomonas may grow even at pH 6.5. This conforms well with the fact that they are observed in soil where the nitrification usually proceeds at a normal rate with rather low pH values (pH 5.5 and lower). Thus, in various soils from Africa—forest soil and the soil of Uganda (pH 5.0-5.5)—grow strains of Nitrosomonas showing an active nitrification process [Kaufmann and Boquel, 1953; Griffith and Manning, 1949]. Paul and Shariff [1954] observed the same thing in a study of the nitrification process in the virgin soils of British Guiana with a pH of 4.5. In acid peat soils, according to the data of Meek and Lipmann [1922] and Flitman [1955], nitrification takes place with a pH of 4.5-4.1. In sand-clay soil with a pH of 4.9 the nitrification of ammonium sulfate lagged behind the nitrification of NH_4OH, which produced very favorable conditions for the activation of nitrifying bacteria [Anderson and Purvis, 1955]. Analogous data were gathered by Birecka, Duda, Kuzminska, and Pedziwilk [1954].

THE ADJUSTMENT OF NITROSOMONAS TO TEMPERATURE

The best temperature for nitrifying bacteria also varies greatly and evidently depends on the ecological nature of the strains being studied. In soils of the extreme north and south (the tundra soils of Novaya Zemlya, various Arctic and Antarctic soils) strains of Nitrosomonas live at the optimum reduced temperature (4-6-11°) [Isachenko and Simakova, 1934; Kazanskii, 1932; Ruban, 1960]. According to Sakai [1959] and Frederick [1957], nitrification at low temperatures (2C, 5C, 15C) is inhibited most in soils with a small number of nitrifying bacteria and a low ni-

trifying capacity may be observed at low temperatures only in soils enriched with nitrifiers. The best temperature for nitrifying bacteria isolated from various soils of temperate countries is in the range of 24-28.5 C, occasionally 29-32 C [Winogradsky and Omelyanskii, 1899; Nelson, 1931; Lees, 1952; Meiklejohn, 1954; Lozinov and Ermachenko, 1959; Ruban, 1960]. Pangamiban [1925], Tandon and Dhar [1934] point out that nitrifying bacteria living in the soils of tropical countries grow best at 35 C.

According to Ruban's data [1960] cultures of Nitrosomonas produced from the filters at the Chelyabinski power plant had an optimum of 40 C. Boullanger and Massol [1903, 1904], Warington [1891], Gibbs [1919], Meiklejohn [1954], Mishushtin [1950] note that autotrophic nitrifying bacteria can not grow above 50 C. Thermophile nitrifiers capable of growing naturally at high temperatures are not found. Therefore, in such natural environments as hot springs Nitrosomonas is not found.

ADJUSTMENT OF NITROSOMONAS TO CONCENTRATIONS OF INORGANIC SUBSTANCES IN ITS ENVIRONMENT

A number of authors have noted the presence of the nitrification process in sopochnykh* and medicinal earths in the estuaries and muds of lakes [Rubenchik, 1929; Rubenchik and Giokherman, 1935, 1939, 1940, 1941; Tutaeva and Monosova, 1946]. In samples from bottom sediments of lake estuaries and muds there were, according to their data, from 10 to 10^3 nitrifying bacteria. Rubenchik isolated the variant Nitrosomonas europaea Winogradsky from a culture which had almost coccus-like bacilli 1.2-1.7 μ in length and 0.9-1.1 μ in width from the saline mud of the Kuyal'nitskii estuary. The strain consisted of halophile organisms and did not grow without NaCl, KCl and $MgCl_2$ in its environment. The optimum NaCl concentration was 5%, minimum – 1%, and the maximum – 15%. In the 5% NaCl concentration the halophile Nitrosomonas accumulated up to 950 mg/liter N_2O_3 in a 40-day test. Peptone and glucose appeared in the halophile strain of Nitrosomonas, depressing the effect in concentration by 0.2%. It may be stated that in an estuary, evidently, organic matter is present in very small amounts and therefore Nitrosomonas strains are not adaptable to the endurance of high concentrations of these substances. Observation of different salt reservoirs establishes the adaptability of nitrifying bacteria to growth in these environments. Even in the strong distillation of Kuyal'nitskii estuary water halophiles remain, growing better in 2-3% NaCl than without its addition. Isachenko [1925, 1938] mentions the presence of nitrifying bacteria in sea water, i. e., in 34% concentration of NaCl. The strains are highly specific and do not grow in fresh water. Kriss and Rukina [1949] found nitrifiers in the hydrosulfide soils of the Black Sea region in quantities of 1,000 bacteria per 1 g of mud. According to the observations of Isachenko [1938] and Kriss and Rukina [1949] the nitrification process proceeds vigorously in mineral environments with sea water. The works of a number of foreign authors [Carey, 1938; ZoBell, 1946; Waksman, Hotchkiss and Carey, 1933; Carey and Waksman, 1934] also indicate the presence of nitrifying bacteria in sea water and in samples of sea mud. They were found at great depths in the sea (to the north of Bermuda at depths of 4,742 m) regardless of the distance from shore and the depth of the station. According to the data from Vargues [1957] the mud from coastal sea zones contains nitrifying bacteria more hardy to salt than the nitrifiers from soils. All of these studies demonstrate that nitrifying bacteria are natural dwellers of the sea bottom but that they are not carried to the shore. Concerning the "sea" origin of Nitrosomonas the data indicate its predominance in bottom sediments and the presence of an intensive oxidizing reaction in saline environments. Rubel' [1913], isolating Nitrosomonas from the drainage water at the Tsarskoselo Biological Station, found that the nitrification process takes place without difficulty if the NaCl concentration in the environment does not exceed 0.8%.

Nitrosomonas living in water mains in significant quantities – 10-1,000 in 1 ml – and in the gravel filter up to a million cells in 1 ml (Detroit River) [Feben, 1935] were adapted to the endurance of a large amount of chlorine, since the water treated by the filters is first treated with chlorination. Nitrifying bacteria survived in chlorine concentrations of 2 mg/liter and enteric bacilli were already destroyed with a concentration of 0.5 mg of residual chlorine in 1 liter of water.

ADJUSTMENT OF NITROSOMONAS TO CONCENTRATIONS OF ORGANIC MATTER IN ITS ENVIRONMENT

Winogradsky and Omelyanskii [1899] arranged the order of the negative relationship between pure cultures of nitrifying bacteria and the presence of even a minimal concentration of organic matter in the environment (glucose – 0.2%, peptone – 0.2%, asparagine – 0.2-0.3%, sodium acetate – 1.5%, meat broth – 20-40%, etc.). Subsequently a number of other investigators showed that they may endure a significantly higher concentration of organic matter than was determined by Winogradsky and Omelyanskii – up to 4-10% glucose, 20% sucrose, 12% urea, 3% asparagine, 10% glycerol, etc. [Meyerhoff, 1916, 1917; Boltjes, 1935; Nelson, 1931; Gibbs, 1919 and others].

From an ecological point of view these results are not contradictory. Observation indicates that in soils rich in organic matter, in manure piles, and in sewer water the nitrification process is more intensive than in substrates poor in organic matter. It was also found that in substrates with a high content of easily assimilated organic matter the nitrifier cells are not killed but only retarded in the nitrification process, which is initiated by a lowered concentration of organic matter to a specific level at the expense of the activity of heterotrophic bacteria [Lapshin and Stroganov, 1936; Klyueva, 1940; Boltjes,

* Transliteration of Russian–Publisher.

1935; Lees and Quastel, 1946; Quastel and Scholefield, 1949].

In substrates with a high content of various organic substances nitrifying bacteria also have a number of characteristics. Here, it is first of all necessary to check Jansen's work [1959]. The author isolated a Nitrosomonas strain from farm manure and studied its morphology and physiology in connection with its environment. It is known that manure is very rich in various organic compounds and is a completely favorable environment for the growth of many microorganisms. The large number of ammonificators contained in manure provides nitrifying bacteria with the necessary source of nitrogen and energy--ammonia. The nitrification process in manure begins, as was shown by Waksman, Cardou and Hulpoi [1939], at 28 and 50 C, and after that, since they are all easily decomposable, the organic compounds will be decayed by the microorganisms. This takes place in approximately a month from the beginning of manure decomposition in composts and, according to the data of Mal'tseva [1955], in four months when the heat method of composting is used. The strain of Nitrosomonas isolated by Jensen was similar in its morphology to Winogradsky's description of Nitrosomonas europaea, but it did not form zoogloea. This strain nitrified ammonium sulfate in the presence of a high concentration of various organic compounds--carbohydrates, sodium salts of various organic acids, various nitrogenous organic compounds, glycerol, ethanol and others. Thus, for example, the Nitrosomonas isolated by Jensen may still grow in the presence of 10% glucose in the environment (filtered). Autoclaved glucose checked nitrification in concentrations of 1.5-2.5%. Depression of the nitrification process in pure cultures of Nitrosomonas by the addition of various carbohydrates (xylose, galactose, glucose) and organic acids (malic, lactic, citric) to Winogradsky's medium was observed by Ruban [1960]. The number of Nitrosomonas cells in the medium with a high glucose concentration decreased together with a decrease in the number of nitrites. It also appeared possible to use the amino group of derived purines (allantoin, guanine, and urea). Thus, guanine is converted into xanthine in Nitrosomonas cultures and in this way the amino group undergoes nitrification. Ruban, Rylkina and Pokrovskii [1957] showed that sucrose in concentrations of 50 mg in 10 ml of medium inhibited the growth of Nitrosomonas and the formation of nitrogen.

According to Neelova [1903] Nitrosomonas isolated from fertile garden soils withstands large concentrations of cane sugar (up to 10%), lactose (6%), galactose (4%), fructose (2%), glucose (5%), soluble starch (2% and higher), various alcohols, etc. In sewer water, where the separate composite parts are soaps, fats, and some disinfecting substances (phenol), nitrifying bacteria adapted to life under those environmental conditions live [Rubel', 1913]. A pure culture of Nitrosomonas isolated by the author from oxidized coke at the Tsarskoselo Biological Purification Station withstood up to 0.3% oleic acid Na and 0.01% phenol. A culture of nitrifying bacteria taken for comparison from soil had already stopped oxidizing ammonia in the presence of 0.005% phenol.

The nitrifying bacteria of sewer water differed even among themselves in morphological characteristics. They were small in size (0.5-0.6 μ) and were immobile. In gel they grew in the form of very small gray-blue colonies which, under the microscope, had a brown color. The nitrification process was very intense in the air tanks [Chick, 1906; Winogradsky, 1937; Remezova, 1938]. The oxidation of ammonium nitrogen begins in the air tanks, as the work of Bazyakina [1923, 1936] shows, when dissolved oxygen forms (reduction BPK) in the aerated mixture of sewer liquids and active sludge. The major factors in determining the rate of the oxidation process in air tanks are the intensity of aeration and the amount of active sludge. The optimal concentration of active sludge is 26-33% of the total volume of the aerated mixture. The pH value of sewer water has also been shown to be stable (7.6-7.8). The high buffer action of sewer liquid and the presence in it of calcium and magnesium bicarbonates produces favorable conditions of acidity for the growth of nitrifiers. When there is a deficiency of Ca and Mg in the sewer liquids nitrification leads to an abrupt acidification of the liquid and a depression of further activity by the nitrifying bacteria. According to Kovshikova [cited by Remezova, 1938] a pH value in a laboratory model air tank up to 7.1-8.5 does not influence the rate of nitrification. At pH 7.0 the rate of nitrification diminished by 30% and at pH 5.8 the process dropped off by 94%.

Remezova notes that under ideal conditions in a laboratory air tank nitrification may take place with unusual intensity: the rate of the process may reach 14.7 and 29 mg of nitrogen per hour in 1 liter. The nitrites in the environment do not exceed 13% of the total amount of oxidized nitrogen in the air tank. The number of nitrifying bacteria themselves in the active sludge is on an average of 20 million (from 11 to 32 million) in the conversion of 1 g dry matter of active sludge. With a sludge concentration of 20-22% after 30 min of settling in 1 ml of the mixture of active sludge and purified liquid, 114-148 thousand nitrifying bacteria were counted [Remezova, 1938]. Hence the unusual number of Nitrosomonas in active sludge is quite clear. According to Winogradsky the nitrifying flora of active sludge is characterized by a large variety of forms in comparison with the nitrifying flora of soil.

Free forms--Nitrosomonas--had rather large, short bacilli. In gel they produced colonies with a yellow-orange color which became brown with age. Zoogloea forms, for example Nitrosogloea, in gel produced colonies from thick zoogloea formed of cocci tightly knit as a single film (the predominant nitrifier form in sludge). Remezova [1938] in working with the active sludge did not find the zoogloea forms described by Vinogradskaya

[1937]. Vinogradskaya also pointed out that the nitrifying activity of sludge becomes stronger with the introduction of iron. Boswell and others [1954] indicated that the Nitrosomonas from sewer water grow in pure cultures in the presence of peptone, yeast extracts, and glucose. Peptone and yeast extracts in concentrations of 0.00000001 are not effective, but 0.0000001 already shows a substantial effect on the change of its speed. Glucose in concentrations of approximately 0.00002 does not cause a change in the rate of growth. The optimal conditions for the growth of this strain of Nitrosomonas in the laboratory are: an ammonium nitrogen content of 0.0000005, pH 8.0-8.5, and a temperature of 32C.

All the material mentioned above indicates that nitrifying bacteria have a high adaptability to varying conditions in the external environment, in particular, they easily adapt to the concentration of mineral and organic matter, an active reaction of the environment, temperature and to a number of other factors. Therefore, in various soil types, active sludge, manure, estuaries, and sea water, ecological strains of Nitrosomonas with specific physiological and morphological characteristics are found. Nevertheless, their relationship to ecological conditions has, up to the present, been poorly studied. The strengthening of study of the ecology of specific groups of bacteria ought to be given considerable attention in the future.

LITERATURE CITED

1. N. S. Avdonii, I. P. Arens and L. N. Stepanova, Pochvovedenie, 9, 25 (1960).
2. N. A. Bazyakina, Tr. Soveshch. po ochistke stoch. vod pri kanalizats. otdele M. K. Kh. 1, No. 3(1923).
3. N. A. Bazyakina, The Oxygen Balance in Air Tank Performance in Full Purification [in Russian](Moscow, Izd-vo "Vlast' Sovetov," 1936).
4. E. F. Berezova and E. Kh. Rempe, Tr. Vses. n.-i. in-ta s.-kh mikrobiol. 15(1958).
5. V. N. Beresneva and O. I. Shvetsova, Tr. Otd. s.-kh. mikrobiol. 3 (1928).
6. G. Burgvits, Tr. Otd. s. -kh. mikrobiol. 3 (1928).
7. G. Burgvits, Tr. Otd. s. -kh. mikrobiol. 4 (1930).
8. E. I. Butylin, Mikrobiologiya 5, No. 1 (1936).
9. V. N. Bylinkina, Tr. Otd. s.-kh. mikrobiol. 3 (1928).
10. F. P. Vavulo, The Microflora of Peat Bog Soils and their Changes under the Influence of Development and Agricultural Use [in Russian] Author's abstract dis. (Minsk, Belor. un-t, 1955).
11. E. Winogradsky, Ann. Inst. Pasteur 58 (1937).
12. S. N. Winogradsky, Soil Microbiology [in Russian] (Moscow, Izd. AN SSSR, 1952) Chap.: "Nitrification," p. 145.
13. S. N. Winogradsky and E. S. Winogradsky, Ann. Inst. Pasteur 50, 350 (1933).
14. S. N. Winogradsky and V. L. Omelyanskii, Arkh. biol. nauk 7, No. 3 (1899).
15. E. A. Voroshilova, Mikrobiologiya 23, No. 1 (1954).
16. L. A. Garder, Tr. Otd. s.-kh. mikrobiol. 3, 183 (1928).
17. L. A. Garder, Tr. In-ta s.-kh. mikrobiol. 4 (1930).
18. I. F. Garkusha, Pochvovedenie, No. 10, 19 (1953).
19. P. A. Genkel' and E. I. Butylin, Mikrobiologiya 4, 3, 363 (1935).
20. P. A. Genkel' and E. M. Danini, Mikrobiologiya 5, 1, 99 (1936).
21. F. M. Germanov, Pochvovedenie, No. 3 (1933).
22. V. T. Glotova, Pochvovedenie, No. 8 (1950).
23. E. M. Danini and V. A. Kosmortov, Mikrobiologiya 2, No. 1 (1933).
24. E. A. Domracheva, Tr. SOPS AN SSSR, ser. zakavkazsk. 2 (1933).
25. L. M. Dorosinskii, Mikrobiologiya 23, No. 5 (1954).
26. R. A. Zhukova, Mikrobiologiya 25, 5, 569 (1956).
27. A. A. Imshenetskii, Microbiological Processes at High Temperatures [in Russian](Moscow-Leningrad, Izd-vo AN SSSR, 1944).
28. A. A. Imshenetskii, Dokl. AN SSSR 47, No. 3 (1945).
29. A. A. Imshenetskii, Tr. konf. po napravl. izmenchivosti i selektsii mikroorganizmov (Moscow, Izd-vo AN SSSR, 1952).
30. A. A. Imshenetskii, Mikrobiologiya 24, No. 4 (1955).
31. A. A. Imshenetskii, Mikrobiologiya 25, 3, 330 (1956).
32. B. L. Isachenko, Tr. I Vses. gidrol. s'ezda, p. 495 (1925).
33. B. L. Isachenko, Mikrobiologiya 7, No. 4 (1938).
34. B. L. Isachenko and T. L. Simakova, Tr. Arktich. in-ta 9, 107 (1934).
35. A. F. Kazanskii, Tr. Polyarn. Komissii Akademii nauk 7, 79 (1932).
36. E. K. Kardo-Sysoeva, Tr. Otd. s.-kh. mikrobiol. 3 (1928).
37. B. A. Keller and A. F. Karel'skaya, Priroda i sel'sk. kh-vo zasushlivo-pustynnykh oblastei SSSR, No. 1-2 (1926).
38. N. T. Kiseleva, Materialy VIII sessii AN TurkmSSR, Ashkhabad, p. 26 (1955).
39. I. A. Kiseleva, Tr. Vses. n.-i. in-ta s.-kh. mikrobiol. 15 (1958).
40. L. I. Klyueva, Tr. In-ta fiziol. rastenii im. K. A. Timiryazeva 3, No. 1 (1940).
41. V. A. Kovda and S. N. Selyakov, Priroda, No. 5 (1935).
42. B. A. Kolcheva, The Microbiological Characteristics of the Basic Soil Types of Bulgaria [in Russian] Diss. (Inst. mikrobiol., AN SSSR, 1954).
43. K. A. Kozlov, Uch. zap. LGU, 216, 125 (1956).
44. M. M. Kononova, Tr. Ak-Kavakskoi opytno-orosit. stantsii, Vol. 7, Sb. rabot mikrobiol. chasti, p. 18 (1930).
45. M. P. Korsakova, Tr. In-ta s.-kh. mikrobiol. 4, 1, 3 (1930).

46. S. P. Kostychev and I. S. Kholkin, Tr. In-ta s.-kh. mikrobiol. 4, 1, 71 (1930).
47. S. P. Kostychev, Tr. In-ta s.-kh. mikrobiol. 4, 1, 29 (1930).
48. A. E. Kriss, Mikrobiologiya 16, 5, 437 (1947).
49. A. E. Kriss and E. A. Rukina, Mikrobiologiya 18, 5, 402 (1949).
50. N. A. Krasil'nikov, Tr. In-ta mikrobiol. AN SSSR, 5, 107 (1958).
51. S. I. Kuznetsov, Tr. In-ta mikrobiol. AN SSSR, 4, 130 (1955).
52. T. B. Levinskaya and I. S. Mamicheva, Arkh. biol. nauk 43, No. 2-3 (1936).
53. N. M. Lazarev, Tr. Vses. n.-i. in-ta s.-kh. mikrobiol. za 1941-1945 gg., No. 1, Sel'khozgiz, p. 5 (1949).
54. M. I. Lapshin and S. N. Stroganov, The Chemistry and Microbiology of Drinking Water and Sewer Water [in Russian](Gosstroiizdat, 1936).
55. L. G. Loginova, The Physiology of Experimentally Produced Thermophile Yeasts [in Russian](Moscow, Izd-vo AN SSSR, 1960).
56. A. B. Lozinov and V. A. Ermachenko, Mikrobiologiya 28, 6, 835 (1959).
57. A. B. Lozinov and V. A. Ermachenko, Mikrobiologiya 29, 4, 523 (1960).
58. G. Lopatina, Tr. Otd. s.-kh. mikrobiol. 3 (1928).
59. G. Lopatina, Tr. In-ta s.-kh. mikrobiol. 4, No. 1 (1930).
60. N. N. Lyalikova, The Physiology and Ecology of Thiobacillus ferrooxidans in Connection with its Role in the Oxidation of Sulfide Ores [in Russian] Author's abstract diss. (Moscow, In-t mikrobiol. AN SSSR, 1959).
61. N. I. Mal'chevskaya, Zap. Pushkinsk. s.-kh. in-ta. 8, 140 (1938).
62. N. I. Mal'chevskaya, Pochvovedenie, No. 3 (1933).
63. I. M. Mal'tseva, Tr. Ak-Kavakskoi tsentr. agronomich. stantsii, Tashkent (Izd-vo SAGU) p. 78 (1955).
64. A. Ya. Manteifel', A. I. Zhukova and É. K. Dem'yanova, Mikrobiologiya 19, No. 6 (1950).
65. E. N. Mishustin, The Ecological-Geographical Variability of Soil Bacteria [in Russian](Moscow, Izd-vo AN SSSR, 1947).
66. E. N. Mishustin, Thermophile Microorganisms in Nature and Practice [in Russian](Moscow, Izd-vo AN SSSR, 1950).
67. E. N. Mishustin and M. I. Pertsovskaya, Microorganisms and the Self-Purification of Soil [in Russian] (Moscow, Izd-vo AN SSSR, 1954).
68. N. S. Mitrofanova, Mikrobiologiya 22, No. 3 (1953).
69. A. I. Naumova, Tr. In-ta mikrobiol. AN SSSR, 3, 166 (1954).
70. V. P. Neelov, Otd. ottisk. iz Arkhiva biol., p. 174 (1903).
71. A. A. Nizova, Pochvovedenie, 10, 96 (1960).
72. E. Nikitina, Tr. In-ta s.-kh. mikrobiol. 4, No. 1 (1930).
73. D. E. Novogrudskaya, "Results and Problems of an Investigation on the Role of Microorganisms and their Products in the Life-activity of Food Plants," Tezisy dokl. soveshch. (Moscow-Leningrad, 1959).
74. V. N. Novikova, The Physiology of Nitrification Inducers. An Essay on the History of the Problem [in Russian] Diss. (Moscow, In-t istorii estestvoznaniya i tekhniki AN SSSR, 1960).
75. A. A. Obraztsova, Izv. AN SSSR, ser. biol., No. 1 (1936).
76. Yu. A. Orfanitskii, M. A. Fedchenko and M. Ya. Ardashev, Pochvovedenie, 10. 96 (1960).
77. A. K. Panosyan, The Microbiological Characteristics of the Solonchaks of the Armenian SSR in Connection with Problems of their Utilization [in Russian](Erevan, Izd-vo AN ArmSSR, 1948).
78. A. N. Pertseva, Dokl. Vses. akad. s.-kh. nauk im. V. I. Lenina, 6, 19 (1950).
79. V. É. Pontovich, Tr. In-ta fiziol. rastenii im. K. A. Timiryazeva 7, 1, 51 (1960).
80. P. E. Prostakov and V. P. Mina, Tr. Gorskogo s.-kh. in-ta, 5, 63 Dzhaudzhikau (1945).
81. O. I. Pushinskaya, Tr. In-ta lesa An SSSR 23, 209 (1954).
82. T. S. Remezova, Investigation of the Nitrification Process in Aeration with Active Sludge [in Russian] (Moscow, ONTI, 1938).
83. N. P. Remezov, "The investigation of forest soil science," Tr. VNIILKh, 24, 89 (1941).
84. N. P. Remezov, Pochvovedenie, No. 5 (1951).
85. E. L. Ruban, Mikrobiologiya 25, No. 1(1956).
86. E. L. Ruban, The Physiology and Biochemistry of Nitrifying Microorganisms (First Stage) [in Russian] Author's abstract Diss. (MGU, 1960).
87. E. L. Ruban and V. A. Ermachenko, Mikrobiologiya 27, No. 4 (1958).
88. E. L. Ruban, S. S. Rylkin and N. V. Pokrovskaya, Mikrobiologiya 26, No. 3 (1957).
89. M. N. Rubel', " On the characteristics of microbes in the nitrification of biological filters, "Reprint from Russkogo vracha, No. 18 (1913).
90. L. I. Rubenchik, Ztbl. Bakt., Abt. II, 77, 1 (1929).
91. L. I. Rubenchik, Priroda,5, 41 (1959).
92. L. I. Rubenchik and D. G. Goikherman, Mikrobiologiya 4, No. 3 (1935).
93. L. I. Rubenchik and D. G.Goikherman, Mikrobiologiya 8, No. 5 (1939).
94. L. I. Rubenchik and D. G. Goikherman, Mikrobiologiya 9, No. 1 (1940).
95. L. I. Rubenchik and D. G. Goikherman, Mikrobiologiya 10, No. 3 (1941).
96. E. V. Runov, Tr. In-ta lesa AN SSSR 15, 182 (1954).
97. S. Severin, Vestn. bakt.-agronomich. stantsii, 15, 16 (1909).

98. S. Severin, Vestn. bakt.-agronomich. stantsii, 15, 16 (1909).
99. G. L. Seliber, Mikrobiologiya 24, 4, 468 (1955).
100. G. L. Seliber, Collected Works of the Russian Microbiologists S. N. Winogradsky and V. L. Omelyanskii [in Russian] (Moscow, Izd-vo Min-va s.-kh. SSSR, 1960).
101. T. L. Simakova, Izv. AN SSSR, ser. biol. 7, No. 1 (1932).
102. V. T. Smalii, Mikrobiol. zh. 11, 4, 25 (1950).
103. N. N. Sushkina, Collected Works in Forestry [in Russian] (Leningrad, Sel'khozgiz, 1931) p. 137.
104. N. N. Sushkina, Tr. Pochv. in-ta im. Dokuchaeva, 6, 191 (1932).
105. N. N. Sushkina, Izv. AN SSSR, ser. biol., No. 1 (1933).
106. N. N. Sushkina, Mikrobiologiya 21, No. 1 (1952).
107. V. N. Sukhareva, VNIILM. Byul. nauchno-tekhn. inform., No. 4 (Moscow, 1957).
108. V. G. Taranovskaya and F. G. Germanov, Nosovsk. s.-kh. opytn. stantsiya, 45, 7 (1927).
109. A. S. Tvorogova, Basic Types of Cuttings and their Significance in Forestry [in Russian] (Arkhangel, 1959).
110. A. I. Tutaeva and S. M. Monosova, Mikrobiologiya 15, 3, 195 (1946).
111. M. G. Tyagny-Ryadno, Tr. NIU im. Samoilova, 108, 143 (1933).
112. N. Flitman, Byul. nauk. stud. konf. 1954 r., Part 2, 38 (Lvov, Vid-vo un-tu, 1955).
113. M. V. Fedorov and V. I. Smirnova, Izv. Timiryaevsk. s.-kh. akad., 3, 133 (1953).
114. I. S. Kholkin, Tr. In-ta s.-kh. mikrobiol. 4, No. 1 (1930).
115. I. E. Tsyplenkin and D. G. Zhilin, Khimizatsiya sots. zemledeliya 5, 59 (1936).
116. O. I. Shvetsova, Tr. In-ta s.-kh. mikrobiol. 4, 1, 133 (1930).
117. O. I. Shvetsova, Tr. Vses. s.-kh. mikrobiol. 4, 3, 11 (1931).
118. A. M. Sheloumova and V. A. Berg, Tr. Otd. s.-kh. mikrobiol. 3 (1928).
119. A. M. Sheloumova and V. A. Berg, Izv. AN SSSR, ser. biol., 673 (1931).
120. A. M. Sheloumova and V. Faerman, Tr. Otd. s.-kh. mikrobiol. 3, 3 (1928).
121. G. Shtobe, Sb. "Pochva i urozhai" In-ta pochvoved. i zemledeliya 2, 175 (Riga, 1952).
122. O. Shul'gina, V. Beresneva and S. Norkina, Tr. In-ta s.-kh. mikrobiol. 4, 2, 3 (1930).
123. I. Shulov, "The presence of nitrifying bacteria in normal sandy plant soils," Reprint from Dokl. prochitannogo na II Mendeleevsk. s' ezde (1911).
124. V. S. Shumakov, Pochvovedenie, No. 4 (1948).
125. V. S. Shumakov, Pochvovedenie, No. 10, 47 (1960).
126. N. V. Yashnova, Tr. NIIU im. Samoilova, No. 76, 50 (1930).
127. O. Anderson and E. Purvis, Soil Sci. 80, 4, 313 (1955).
128. H. Birecka, I. Duda, K. Kuzminska, and Fr. Pedziwilk, Roczniki nauk Rolniczych, A-1, 69, 57 (1954).
129. T. Boltjes Kingma, Arch. Mikrobiol. 6, H. 1, 79 (1935).
130. H. Bömeke, Arch. Mikrobiol. 10, H. 4, 385 (1939).
131. I. Boswell and J. Sheldon, Soils and Fert. 14, 2103, 460 (1951).
132. Boullanger and Massol, Deux memoires, Annales 1, 17 (1903).
133. Boullanger and Massol, Deux memoires, Les Annales 2, 18 (1904).
134. A. Buswell, T. Shiota, N. Lawrence and I. Van Meter, Appl. Microbiol. 2, 1 (1954).
135. E. Calder, Soil Sci. 8, 1, 60 (1957).
136. C. Carey, J. Mar. Res. 1, 291 (1938).
137. C. Carey and S. Waksman, Science 79, 349 (1934).
138. P. Chandra and W. Bollen, Appl. Microbiol. 8, 1, 30 (1960).
139. F. Chase and G. Baker, Canad. J. Microbiol. 1, 1, 45 (1954).
140. H. Chick, Proc. Roy. Soc. 77, ser. B, 241 (1906).
141. Ed. Chodzički, Ekol. Polska 2, N. 4, 327 (1954).
142. O. Coppier and H. de Barjac, Ann. Inst. Pasteur 83, 118 (1952).
143. M. Engel and M. Alexander, J. Bact. 76, 2, 217 (1958).
144. D. Feben, J. Amer. Wat. Works Ass. 27, 4, 439 (1935).
145. D. Feher, Abstracted in Mikrobiologiya 16, 6, 555 (1946).
146. G. Fraps and A. Sterges, Soil Sci. 34, 5, 353 (1932).
147. L. Frederick, Soil Sci. 38, 6, 481 (1957).
148. T. Gaarder and O. Hagem, Ztbl. Bakt. 57 (1922).
149. S. Garrett, Trans. Brit. Mycol. Soc. 38, 1, 1 (1955).
150. F. Gerretson and H. deHoop, Canad. J. Microbiol. 3, 359 (1957).
151. W. Gibbs, Soil Sci. 8, 6, 427 (1919).
152. J. Golebiowska and M. Falkowski, Soils and Fert. 16, 2, 546 (1953).
153. Y. Griffith and H. Manning, Trop. Agric. Trin. 26, 108 (1949).
154. T. Hofman and H. Lees, Biochem. J. 52, 4, 19 (1952).
155. T. Hofman and H. Lees, Biochem. J. 54, 4, 579 (1953).
156. H. Jensen, Nature 165, 4207, 974 (1959).
157. I. Kaufmann and G. Boquel, Ann. Inst. Pasteur, 3, 365 (1953).
158. R. Klein, Nature 175, 4450, 292 (1955).
159. H. Lees, Biochem. J. 52, 1, 134 (1952).
160. H. Lees, VI Congr. Intern. Microbiol. (1953) Vol. 3, sect. XVII-XXII.
161. H. Lees, Fourth sympos. Soc. Gen. Microbiol. 84 (1954).
162. H. Lees and J. Quastel, Biochem. J. 40, 5-6, 824 (1946).

163. M. Mason-Williams, Ann. New Scientist 6, 138, 50 (1959).
164. C. Meek and C. Lipmann, J. Gen. Physiol. 5, 195 (1922).
165. J. Meiklejohn, J. Gen. Microbiol. 4, 2, 185 (1950).
166. J. Meiklejohn, J. Soil Sci. 4, 1, 59 (1953).
167. J. Meiklejohn, Fourth Sympos. Soc. Gen. Microbiol. 68 (1954).
168. O. Meyerhoff, Pflüg. Arch. ges. Physiol. 164, 253 (1916).
169. O. Meyerhoff, Pflüg. Arch. ges. Physiol. 166, 240 (1917).
170. D. Nelson, Ztbl. Bakt., Abt. II, 83, 280 (1931).
171. E. Pangamiban, J. Amer. Soc. Agron. 17, No. 1 (1925).
172. H. Paul and M. Shariff, J. Agric. Sci. 44, 377 (1954).
173. J. Quastel and P. Scholefield, Nature 164, 1068 (1949).
174. H. Rehm, Zh. Biologiya 1956, 10, 39073, 68 (1954).
175. H. Sakai, Soil and Plant Food 4, 4, 159 (1959).
176. A. da Silva, Soils and Fert. 15, 1311, 257 (1952).
177. I. Stevenson, Soil Sci. 75, 3, 225 (1953).
178. I. Stevenson and F. Chase, Soil Sci. 76, 2, 107 (1953).
179. I. Szabo and M. Marton, Agzokem es talaj. 7, 3, 287 (1958).
180. S. Tandon and N. Dhar, Soil Sci. 38, 3, 183 (1934).
181. H. Thornton, Proc. Roy. Soc. 145, B, No. 920, 364 (1956).
182. L. Turk, Soil Sci. 47, 6, 425 (1939).
183. L. Varga and P. Dyurko, Magyar. tud. akad. agriatud. Oszt. közl. 6, 1-2, 25 (1955).
184. H. Vargues, Bull. Trav. Stat. Agric. et Pêche Castiglione, No. 8, 59 (1957).
185. S. Waksman and M. Madhok, Soil Sci. 44, 5, 361 (1937).
186. S. Waksman, T. Cardou, and V. Hulpoi, Soil Sci. 47, 2, 83 (1939).
187. S. Waksman, M. Hotchkiss and C. Carey, Biol. Bull. 65, 137 (1933).
188. R. Warington, J. Chem. Soc. 59, 484 (1891).
189. C. ZoBell, Marine Microbiology. A Monograph on Hydrobacteriology (Waltham, Mass., 1946).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. ***Some or all of this periodical literature may well be available in English translation.*** **A complete list of the cover-to-cover English translations appears at the back of this issue.**

THE RUSSIAN MICROBIOLOGISTS S. N. WINOGRADSKY AND V. L. OMELYANSKII

Izd. min-va sel'sk. kh-va, Moscow, 1960

Reviewed by A. A. Imshenetskii

Translated from Mikrobiologiya, Vol. 30, No. 3, pp. 565-566, May-June, 1961

The ever-increasing importance of microbiology as a science impels us more and more often to recall the names of those outstanding scholars to whom microbiology owes so much. The brilliant talent of S. N. Winogradsky enriched our country's science with outstanding discoveries and the successful development of his ideas by V. L. Omelyanskii further consolidated the progress of microbiology in our country. The publication of a collection of papers dealing with the work of our two eminent microbioligists is a happy event for us all. We can only welcome the initiative of the Leningrad scholars who prepared and published these essays. The small collection has 84 pages and contains the following items: a short introduction (G. L. Seliber), "Sergei Nikolaevich Winogradsky, His Life and Work" (N. M. Lazarev), "The Life and Scientific Work of Vasilei Leonidovich Omelyanskii" (Z. G. Razumovskaya), "S. N. Winogradsky and the Ecological Trend in Microbiology" (G. L. Seliber), "Methods of Soil Microbiology Research in the Works of S. N. Winogradsky and Their Later Development" (V. N. Bylinkina), "The Nitrification Studies of S. N. Winogradsky and V. L. Omelyanskii" (V. N. Beresneva), "Studies of S. N. Winogradsky and V. L. Omelyanskii on Aerobic Nitrogen Fixation" (M. Z. Shklyar), "Studies of S. N. Winogradsky and V. L. Omelyanskii on Aerobic Nitrogen Fixation by Microorganisms" (G. V. Lopatina and S. P. Norkina), and "Studies of V. L. Omelyanskii and S. N. Winogradsky on Cellulose Decomposition" (M. S. Loitsyanskaya).

The most noticeable feature emerging from a perusal of the contents of this collection is that in most of the essays the studies of Winogradsky and Omelyanskii are treated as a single whole, inseparable not only as regards content, but also as regards investigational methods, trend of research and the theoretical views of the authors. This can hardly be regarded simply as a continuation of research, since the pupil carried on his research in the years when his teacher had temporarily ceased his investigations, while the teacher worked on for many more years after his pupil had died. Despite this, the studies of Winogradsky and Omelyanskii are one in spirit and belong to the same school, in the best sense of this term. The attempt of the authors of these essays to visualize Vinogradskii's and Omelyanskii's work as a single interconnected whole is perfectly justified and is unquestionably responsible for the great value of this collection.

The second, and again a very important, feature of the collection of essays lies in the exceptionally careful attitude toward the scientific legacy of our eminent microbiologists. This is seen in the very exact reproduction of the content of their works and the preservation of the sense of objectivity and harmony. To retain this latter feature and to communicate the content of a few papers selected from the vast scientific legacy of a scientist is by no means any task. This selection of works and the analysis of their scientific value have been carried out with great knowledge of this particular area of microbiology and has been very successful because the authors who have written about the studies devoted to specific problems are themselves authorities on these problems.

The third, and most important (particularly for young specialists), feature of the essays lies in the fact that in several of them the authors have not confined themselves to an account and appraisal of Winogradsky's and Omelyanskii's studies, but have endeavored to analyze the subsequent development of their ideas and the factual data amassed in recent years in these areas of microbiology. Thus, these articles are not merely historical articles dealing with the work of the founders of our country's microbiology, but are reviews of the development of microbial ecology, the science of nutrition, the problem of nitrogen fixation, and so on. By introducing new information and new scientific facts into these essays the authors have been able to make a contemporary assessment of the value of Winogradsky's and Omelyanskii's studies for the development of microbiology. The authors reveal what has become even more valuable with the advance of science and what has had less influence in the subsequent "forward march" of science. Such a retrospective appraisal is not only of interest from the historical viewpoint, but it also helps in prediction of the development of various fields of science and in the search for the most important lines of research. It is enough to mention the brilliant realization of Omelyanskii's ideas of synthesizing ability of the "little laboratories" of microbes. The obtention of such biologically active substances as antibiotics, enzymes, vitamins, hormones, alkaloids, etc., just

as the wide employment of indicator cultures for analytical purposes, is presumably just the first step in the "triumphal march" of microbes as chemical reagents. As regards the content of the papers, it should be noted that not all the essays give an equally full and deep treatment of the later experimental development of the specific problem. In particular, the treatment of biological nitrogen fixation is incomplete. It would have been desirable to find references to the wide occurrence of nitrogen-fixing ability among lower plants – algae and actinomycetes, the most recent information on the chemistry of fixation, particularly nitrogen fixation by cell-free preparations, and the nitrogen-fixing ability of oligonitrophilic bacteria and its importance for increasing soil fertility. Such references would naturally have to be very brief, since no one can pretend that essays dealing mainly with the work of our country's microbiologists could at the same time be complete reviews of the modern literature on a particular question.

The collection has no serious faults but mention must be made of some small defects that leave an unpleasant impression. In N. M. Lazarev's paper an ambiguous transliteration of a microbiologist's name appears several times. It is quite clear from the context that only on one occasion is the American investigator H. Konn meant, while on other occasions the German scientist F. Cohn is referred to. On p. 49, there appears the phrase "in nitrifying bacteria and assigning them to the group of autotrophs." The context demands "heterotrophs" instead of "autotrophs."

On p. 50 the name "N. B. Zavarzina" is given; "G. A. Zavarzin" is obviously intended. On pp. 61-62 the possibility of Azotobacter utilizing cellulose decomposition products and root excretions has been undervaluated in the discussion of energy sources for Azotobacter.

The collection contains a list of cited literature, but a short list of literature on the life and work of Winogradsky and Omelyanskii would have been desirable. This small volume is printed on good paper and contains two very good portraits of our scientists.

The publication of this collection is a welcome event for all our lovers of the science of the invisible. It is of equal interest and value for the new young research worker and the established investigator. The former will gain an idea of the value of the studies of our country's microbiologists and of a whole stage in the history of microbiology, while the latter will again be convinced of how original methods in the hands of a talented investigator can lead to discoveries which increase in value with the years. Wide circles of Soviet microbiologists will read this book with interest and gratitude.

FYSIOLOGIE A BIOCHEMIE MIKROBŮ

Jiří Stárka

Státní Pedagogické Nakladatelství, Praha, 1959, 491 pages

Reviewed by N. D. Ierusalimskii

Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 566-567, May-June, 1961

This book provides a basic exposition of modern views on microbial physiology and biochemistry. It is an advanced textbook, designed for students in higher courses and research workers with a knowledge of general microbiology and chemistry. The book is divided into seven chapters.

Chapter 1. "The Microbial Cell" contains an account of the form and structure of the microbial cell, including the information obtained by the newest microscopic and electron microscopic techniques about its physical properties (permeability, electric charges, pH of protoplasm, staining, etc.) and about all its important chemical constituents.

Chapter 2. "Growth and Reproduction" is divided into three sections: 1) Growth and division of bacteria, growth phases of culture, relation between growth and nutrition, spore formation, production of filtrable forms, L-cycle, etc.; 2) Reproduction of viruses, including bacteriophage; 3) Effect of physical and physiochemical factors on growth rate and yield of microbial biomass (temperature, radiation, humidity, pressure, pH, oxidation–reduction potential, etc.).

Chapter 3. "Enzymes." Enzymes have been given a special chapter, one of the largest in the book, in view of their decisive importance in metabolism. The first section gives an account of the main properties of enzymes, the nature of enzymatic reactions and their mathematical expression, the effect of various environmental factors, including enzyme poisons, on these reactions, and so on. In another two sections coenzymes and the specificity of enzyme action are discussed. A list of the main groups of enzymes and their properties are given in the next section. The last section deals with the synthesis of adaptive enzymes.

Chapter 4. "Microbial Nutrition." The types of microbial nutrition are classified in this chapter. An account is given of the carbon and nitrogen sources, mineral elements, amino acids, and vitamins required by microorganisms.

Chapter 5. "Variation and Heredity" includes two sections. The first gives a short account of dissociation in microbial cultures. The second gives an equally brief account of the main concepts of microbial genetics (mutation, mutagenic factors, natural and artificial selection of mutants, hybridization of yeasts, bacteria, and fungi, development of resistance, etc.).

Chapter 6. "Metabolism" is the longest chapter. It starts with a general account of the methods of studying metabolism. The second section consists of a full discussion of carbon metabolism–various fermentations, the Pasteur effect, aerobic degradation of carbohydrates, the Krebs cycle, the pentose cycle, mechanism of energy transfer, lipoid metabolism, steroid transformation, etc. The third section is devoted to nitrogen metabolism–the transformation of inorganic nitrogen, amino acid metabolism, and the biosynthesis of proteins and nucleotides. The last section deals with the metabolism of autotrophic bacteria.

Chapter 7. "Action of Antimicrobial Substances" contains a concise exposition of the principles of disinfection, chemotherapy, and antibiotic action.

The book is crammed with factual data. There are more than 60 excellent photographs of bacteria, fungi, and viruses, 40 tables, about 80 graphs and diagrams, and almost 400 chemical formulas.

Despite its fair bulk (more than 40 signatures) the book could not provide detailed coverage of such a vast field of knowledge as microbial physiology and biochemistry. Only the general principles are expounded and for more detailed information the reader is referred to the bibliography at the end of each chapter. It should be noted, incidentally, that the author cites numerous Soviet investigators. This is not often observed in other foreign publications.

The book presents all the most up-to-date theoretical ideas. The development of these ideas is dealt with in historical sequence. Whenever the occasion arises, the author refers to the practical value of each particular scientific theory. Hence, the book is a valuable reference manual for microbiologists of every type and for representatives of related disciplines. This publication compares favorably with several other textbooks on microbial physiology and biochemistry (Clifton's well-known manual, for instance) in its broader, one might say more biological, approach to the subject. In particular, considerable attention has been given to the mor-

phology and cytology of microorganisms, and their ecology and phylogenesis are frequently referred to.

A possible fault of the book is its unequal treatment of different areas of the science. There is a very detailed and comprehensive account of cell structure and properties, growth and development, biochemistry of metabolism, and enzymes. Microbial nutrition and the biosynthesis of such metabolic products as antibiotics, vitamins, etc. are not treated so fully. The account of microbial heredity and variation is too brief and too general. However, genetic questions need not have been discussed at all in a textbook on microbial physiology and biochemistry.

On the whole, the book must be regarded as very successful. Its publication is an important contribution to the world literature furthering the spread of knowledge of physiology and biochemistry. It is a pity that a Russian translation of the book is not planned and hence it will be inaccessible to wide circles of Soviet readers.

PROBLEMS IN MICROBIOLOGY AT THE XV INTERNATIONAL CONGRESS OF THE MILK INDUSTRY IN LONDON

A. Skorodumova

Translated from Mikrobiologiya, Vol. 30, No. 3, pp. 568-571, May-June, 1961

The XV International Congress of the Milk Industry met in London during June-July, 1959. Approximately 3,000 delegates were present. The USSR delegation which took part in the proceedings of the Congress consisted of 56 men.

At the Congress, 380 reports were presented, of which 18 were reports of Soviet scientists and specialists, and of these three reports were on the microbiology of milk products. The reports presented at the Congress were issued in four volumes and were distributed to all delegates to the Congress one and a half months before its inception.

After the solemn opening ceremonies and general reports, the work of the Congress was carried out in six sections; in three of these—new methods of milk treatment, the technology of milk products, and chemico-bacteriological control in production and methods of research—microbiological questions were considered.

NEW METHODS OF MILK TREATMENT

Methods of milk treatment directed toward the elimination or checking of bacterial growth in milk may be divided into the following groups: thermal treatment, use of preservatives and antibiotics, freezing, centrifuging, treatment with electricity, ultraviolet rays and ultraviolet waves and high electron radiation. Some of these were discussed. It was universally recognized that the elimination of bacteria by the treatment of milk with various types of electric current amounted to thermal action. Hamsolt, examining the effect of ultrasonic waves, arrived at the conclusion that the sensitivity of different species of bacteria varies significantly and that it is not possible to consider the results of such milk treatment satisfactory. Berton, in a report on milk treatment by ultraviolet exposure, notes an increase in vitamin D content in the milk and the elimination of microflora, but this method did not have a practical use. In a seminar treatment of milk through the use of centrifuging was suggested. A special centrifuge producing 300 l/hr at 9,000 rpm was used for the removal of bacteria from milk. The number of microflora was reduced approximately 90%. Investigation of the effect of gamma-rays and high-activity elements used with the goal of preservation showed that a change in the taste quality of the product accompanies the method. During the past ten years, particularly in warm countries, interest has been renewed in the addition of hydrogen peroxide to raw milk of unsatisfactory bacterial quality with the idea of avoiding milk spoilage. Whereas economic conditions are not such as to make it possible to improve the milk quality, the addition of hydrogen peroxide for such regions may be recommended. Overby, in a report concerning the use of antibiotics as milk preservatives, noted that the use of antibiotics ought to be combined with other preservatives.

It was pointed out in a short report on different methods of rendering milk harmless that they all occurred in the processing stage. A continuous method of thermal sterilization of milk in which the milk is heated to 130 C for two seconds and less with subsequent cooling deserves notice. Ogasa and others reported the results of testing such a method under practical conditions. When the number of spores (Clostridium) in the original milk is 1,000 in 1 ml, treatment at 130-135 C with exposure for two seconds guarantees full sterilization of the milk. Milk subjected to treatment of 128 C for two seconds may be preserved after bottling for one day at room temperature or seven days in a refrigerator, but pasteurization at 75 C with a 15-min exposure ensures that the milk will keep less than one day at room temperature and four days in a refrigerator. The basic cause of spoilage in both cases is the normal flora. Etne and Berozher reported that in such cases, when ultra-high temperature treatment is accompanied with sterilization of the milk in bottles, the cause of contamination with thermophile spores is the bottling machine. Addition of nysine to the milk before bottling depresses the development of cells, and the final sterilization in the bottles becomes more effective. Ford and others studied the significance of the high-temperature treatment of milk from the point of view of food value and came to the conclusion that riboflavin, pantothenic acid, nicotinic acid and biotin did not undergo any loss, but thiamin was 40% destroyed and vitamin B_{12} almost completely destroyed by repeated sterilization in bottles.

R. J. MacWalter stated the following conclusion: "The most promising progress in the field of research on the biological stability of milk possessing good taste quality comes from an investigation in the realm of high-temperature, rapid sterilization by a continuous method with subsequent bottling of the milk in small bottles, avoiding repeated contamination. The sterilization of milk without serious loss of taste qualities and food value is achieved sufficiently for practical aims by the heat method, heating it to 130-140 C with a two-second exposure, if the bacteriological quality of the raw milk meets the required standard. The greatest difficulty is found in the final stage of the process – bottling; the means by which bacterial contamination may be avoided in bottling ought to be solved by bacteriologists, mechanics, and technologists together."

From the report made on dry milk it would seem pertinent to consider Howell's research on the question of the presence of staphylococci in the dry milk from a spray desiccator. The first definitely determined outbreak of an epidemic took place in England in 1953. The author believes the formation of toxins by staphylococci in dry milk might have had a double origin: first, if the dry milk already contained a previously generated toxin, or indirectly as a result of staphylococcal propagation after reduction of the milk. According to Howell's observations the strains of staphylococci appearing in dry milk were usually characteristic for a particular plant, where some of them were derived from the servicing actions of the staff. Contamination may come from dust or air in which particles of dry milk contaminated by a small number of staphylococci are contained. The facts experimentally produced and verified by the author, that each plant has its characteristic strain and that contamination by staphylococci usually appears in the final stages of production, deserve attention.

METHODS OF BACTERIOLOGICAL ANALYSIS OF MILK AND MILK PRODUCTS

Swortling (Switzerland) notes in his theoretical report that investigation based on color reduction by the reduction test with methylene blue is somewhat unreliable for use in controlling the bacteriological quality of milk inasmuch as the relation between the time of reduction and bacteriological contents is open to the action of many factors. An important factor in the technique of analysis with methylene blue is the concentration of color. A change in the methylene blue concentration influences the interchange between reduction time and the number of bacteria; it is consequently necessary to standardize the concentration of methylene blue as is done in some countries. The standardization of color is determined by the spectrophotometric method.

In the accepted standard abroad there may not be any coli-aerogenes, that is, the lactose fermentation test must be negative. For several years questions concerning the exposure temperature of the test have been under consideration. English specialists argue that a temperature of 30 C gave a larger number of fermented samples than 37 C. The results of official tests in Northern Ireland showed that at 37 C the test for coli-aerogenes was 8% positive, whereas at 30 C it was 21% positive. Can the milk industry maintain this standard? Murray, presenting material worked out by him, showed that the plant with a high sanitary level can definitely maintain such a standard.

Sibilis investigated the number of microorganisms under paraffin oil. In these tests 13 species of molds, 52 species of yeasts, and 8 of bacteria were observed in specific media under paraffin oil in the course of two years at room temperature. It was determined that the size and also the morphological and physiological characteristics of the types did not change in comparison with parallel cultures which were transplanted each third month.

Among the more graphic methods of bacterial classification were ones based on the current biochemical method of analysis. Some of these were discussed at the Congress; in one of them, work using the principles of Cumins and Harris was explained, i. e., investigation of cell case composition; in others, methods worked out by Mettik and others in which the percentage of amino acids in an extract of whole bacterial calls is analyzed qualitatively with the aid of chromatographs on paper were used. Candler and others introduced a simplification of the original method: instead of the trypsin treatment of cell material they obtained cases free from protoplasm by extraction of them from washed cell material with 1 N NaOH at 60 C for 2 hr. This method completely separates Lactobacterium (Lactobacillus) plantarum from the remaining lactobacilli, since the cell case of Lactobacterium plantarum contains diaminopimelic acid, whereas the cases of other lactobacilli contain lysine. Bottazzi, studying the bacterial content of Italian cheeses of the Gran and Fontin varieties, verifies the fact that the chromatographic method of total cell extracts used by Mettik and others is very costly.

PURE CULTURES (YEASTS)

The cultures used in the composition of yeasts by industry are usually not made public. Therefore, Swortling notes with satisfaction that Espersen, from the Christian Hansen Laboratory, published research issued by that laboratory on the problem of yeast composition. The aroma of yeast is produced by a variant of Streptococcus lactis called Streptococcus diacetilactis, fermented by citric acid. Leuconostoc is rarely found.

Polsch noted a case of poor yeast culture development because of the presence of growth-inhibiting microorganisms in the milk. Khebai and others isolated seven species of microorganisms belonging to serological group N – apparently Streptococcus lactis – and three species which did not react with serum group N in analysis of the precipitate and which showed a certain similarity to enterococci from such milk and from hay going into

fodder for cows. Factors inhibiting growth of species isolated from other sources were not identical. They resisted the inhibiting agent when heated in an acid medium but were readily broken down with heating at pH 7.0-9.0. The substances were treated by dialysis by means of a colloidal film. Cheval and Moquette proposed a simple method for the determination of nysine in milk, in which the coagulation time of rennet was used as a measure of the slight difference in acidification of the lactobacilli in milk containing a known concentration of nysine in comparison with milk containing unknown nysine preparations. The principle of the method will be useful in laboratory practice. The remaining "veterinary" antibiotics may be found in milk in concentrations sufficient to hinder the growth of yeasts and cause serious economic loss, and it is therefore desirable to have a rapid and reliable method of analysis of the occurrence of these antibiotics in milk.

The increased use of refrigerators and the tendency to collect milk every second and third day increased the psychrophilic flora in the milk. Thomas and others determined the number of psychrophilic colonies in seven days at a temperature of 3-5C in the wash water of the milking machine. Cleansing by means of immersion in a 2% solution of NaOH and steaming gave a very low bacteria count, but chemical disinfection gave a higher count. Among the psychrophilic flora in raw milk kept for a period of three days at 3-5C, caseolytic and lipolytic gram-negative bacteria predominated; the number of coli-aerogenes often increased in raw milk in spite of the low storage temperature, which was not controlled in the same manner as in the storage of commercial pasteurized milk. It was determined that damage to pasteurized milk first partially freed from psychrophiles was not appreciable before 14 days' storage under refrigeration. Khiteranta and Forsen investigated the action of various mixed yeasts on yeast quality for butter. It was determined that different yeasts have different conditions for the production of a good product (the grade of the milk, the milk processing, the temperature, the duration of ripening, etc.). For example, a certain degree of viscosity was helpful in yeasts used for butter. For taste quality diacetyl is necessary; volatile acids and carbonic acid improve the taste and aroma.

In the subsection on the utilization of the milk industry's waste products, problems concerning the use of skim milk and cheese whey were resolved. It was mentioned in particular that the production of milk vinegar and alcohol from whey was not profitable; all remarked that a large volume of whey does make alcohol production profitable.

There are in London about ten milk plants manufacturing primarily pasteurized milk with various fat contents—3%, 4%; in these plants are small bacteriological laboratories for determining the total number of microbes in pasteurized milk (from 10,000 to 40,000 in one ml) and for carrying out fermentation tests (there may be no coli-aerogenes in one ml).

The central laboratory of the United Dairies Co. has under its supervision coordination of the work of 60 control laboratories. There are in the laboratory ten sections organized under the headings of bacteriology and microbiology. The bacteriological section is responsible for the methods of bacteriological analysis of the milk, cream, butter, and water with which the plant is provided. Scientific research is centered in the study of microorganisms for cheese (their preparation and propagation), in the problem of bacteriophages in cheese production. The microbiological section develops tests for bacteriological control, determining the quality of milk and other foodstuffs. Scientific research in this section at the current time is concentrated on the action of antibiotics in cheese production and the classification of the cause of bitter taste in cream. The laboratory is well equipped with all the necessary apparatus for making tests on the technology of milk production.

From the Scientific Research Institute we visited the National Institute for Milk Economy Research at Shinfield near Reading. It has a dairy farm and a large number of specialized laboratories (for feeding and metabolism, physiology, bacteriology, chemical microbiology, physics, engineering, study of the nutrition value of milk, vitamins, radiobiochemistry, experiments on the technology of milk products, and others). The following subjects are studied in the bacteriological laboratory: the enteric flora of domestic animals (from the intestines of cattle); antibiotics—stimulants to the growth of hogs, chickens; study of mastitis, its stimulants—streptococci, staphylococci, and their bacteriophages; the basic principles of the classification of lactobacilli in milk and cheese (by serological and chromatography methods); the testing of chemical sterilizing agents; tests indicating errors in pasteurization and sterilization (tests on phosphates); spore-forming bacteria and their differentiation according to biochemical and morphological characteristics; the differentiation of species of the genus Bacillus by morphology of spores using the electron microscope. At the Institute is the national collection of milk product microorganisms (a published list) and also there is organized production of dry cultures of lactobacilli by the lyophilic desiccation method. In the reports of the contributors a study of production of the vitamins in group B both in pure cultures of various species of lactobacilli and in various yeasts yielded negative results. Lactobacilli do not synthesize vitamins of group B; different species of them were used as test objects for vitamins by the biological method. For example, for riboflavin the test object might be Lactobacterium casei, for B_6—Streptococcus faecalis, for folic acid—Lactobacterium casei, Streptococcus faecalis. Test objects for vitamin B_{12} might be Bacterium coli, Achromonas malhamensis, for nicotinic acid—Achromonas malhamensis.

In specific investigations Achromonas malhamensis determines only the active form of vitamin B_{12}, but Bacterium coli, Lactobacterium leichmanii, the analogs ("pseudo-vitamins"). The chemical-microbiological laboratory studies the production of nysine (antibiotic lactostreptococci) and its chemical composition. It is produced in the laboratory in crystalline form. Nysine has not developed a medical use, but unpurified nysine is used in the preparation of melted cheeses. The differentiation of microorganisms by chromatography of cell extracts is also studied in this laboratory and investigations of the ripening conditions of cheeses in small quantities is carried out. Among the literature we obtained at the Congress the only item on microbiology was a small advertisement on "Epras," a preparation consisting of a dry culture of acidophilic cells (Lactobacterium acidophilum). "Epras" is used as a medicinal prophylactic in gastro-intestinal illnesses originating from the intake of antibiotics. The preparation contains species Lactobacterium acidophilum of human origin which are stable toward antibiotics. It is stable to penicillin, streptomycin, chlortetracycline, oxytetracycline, tetracycline, erythromycin, chloramphenicol, and neomycin. This compound inhibits the growth of Staphylococci, Monilia, and Candida albicans. Furthermore, the compound stimulates the growth of children.

THE 60TH ANNIVERSARY OF E. N. MISHUSTIN

Translated from Mikrobiologiya, Vol. 30, No. 3,
pp. 571-572, May-June, 1961

On March 22, 1961, the 60th birthday of Corresponding Member of the Academy of Sciences, USSR, doctor of biological science, professor, and laureate of the Stalin Prize, Evgenii Nikolaevich Mishustin, was celebrated. In the year 1917 E. N. Mishustin matriculated at the Moscow Academy of Practical Commercial Science. In 1919-1920 he served in the ranks of the Red Army and in 1920 he was sent to the Timiryazev Academy, from which he graduated in 1924, having specialized in agricultural microbiology under Professor N. N. Khudyakov.

His scientific work began while he was a student in 1921 under Professor A. F. Voitkevich at the Moscow division of the Institute of Agricultural Microbiology, All-Union Academy of Agricultural Sciences, where he worked until 1931, in the last years occupying the post of director of the laboratory.

From the time of his work in the Institute of Agricultural Microbiology, All-Union Academy of Agricultural Sciences, Evgenii Nikolaevich carried on a series of studies on the microbiology of soils, fodders, and nutritive products.

In 1931-1939, Mishustin worked as a director of the laboratory of microbiology and sterilization at the Institute of Fertilizers and Soil Science, All-Union Academy of Agricultural Sciences (All-Union Fertilizer and Soil Science Institute).

The laboratory of microbiology, All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering, under the direction of Evgenii Nikolaevich, carried out a series of significant investigations on the problems of production and testing of bacterial fertilizer. He took a direct part in the organization of bacterial fertilizer production in Moscow, Kuibyshev, and other cities.

In 1939, and up to the present, E. N. Mishustin has been director of the Department of Soil Microorganisms of the Institute of Microbiology of the Academy of Sciences, USSR, where he successfully worked on a series of important problems in agriculture and, in particular, microbiology. Of great over-all theoretical significance were his studies in the field of ecology, ecological variability, and the classification of spore-producing bacteria, microscopic soil fungi and the groups of microorganisms which decompose soil humus. This work permitted Professor Mishustin to work out an original theory on the specificity of microbe associates in various soils.

He determined that as one moves from north to south the number of soil bacillary forms and the range of the species spectrum increases. A similar rule was also established for Actinomyces. It was shown that mass reproduction of fungi become acclimated to northern soils. It was also shown that the specific expansion of individual species of fungi and spore-producing bacteria was linked with their physiological characteristics.

This research allowed Evgenii Nikolaevich to formulate a statement concerning the possibility of using biological characteristics as an indicator of the variety of biogenesis and age of a soil. Furthermore, the possibility of applying these indicators in sanitary microbiology and grain microbiology was shown.

Under E. N. Mishustin's direction and with his personal assistance a theoretical basis for soil sterilization was developed. He gave considerable attention to the development of a scientific base for soil cultivation. On this problem he gave recommendations and proposals to the Ministry of Agriculture of the USSR and to other organizations.

E. N. Mishustin carried out research on the microbiology of grains and of the products from its processing, on problems concerning the spontaneous heating of grains, and on nutritive microbiology. He initiated research on the cause of molding in butter and other products. He took part in developing a technique for making sour green olives and a system for sterilization of canned food.

E. N. Mishustin interested himself in the work of sanitary microbiology, for the most part in problems of the utilization of urban waste products in agriculture by the establishment of sanitary conditions in an area and its environs. This research allowed him to conduct a series of valuable practical experiments on the technique of rendering waste products harmless, on a method of research for sanitary microbiology, etc.

E. N. Mishustin developed a theoretical system of ensilage, which served as the basis for further research, leading to important collaboration with Professor A. A. Zubrilin. This research was published in specialized monograms, one of which was published as early as 1931, and another more voluminous one in 1960.

E. N. Mishustin is the author of over 200 works, including six monographs. A number of studies by E. N. Mishustin were recognized by prizes and awards. For the monograph "Thermophile Microorganisms in Nature and Practice" [1950] he was awarded the Stalin Prize of the third order. The monograph "The Ecological-Geographic Variability of Soil Bacteria" [1947] received the V. R. Wil-

liams Prize of the USSR Academy of Sciences. For the monograph "Microorganisms and the Self-Purification of Soils" [1954] he was awarded the Williams 1st order prize of the K. A. Timiryazev Agricultural Academy. In 1940 the All-Union Agricultural Exhibition awarded E. N. Mishustin a small silver medal and in 1955 a small gold medal for research in the field of agricultural microbiology.

E. N. Mishustin was awarded the Order of Lenin and the Order of Red Labor Citation and also a series of medals.

E. N. Mishustin took active part in the training of a cadre of microbiologists. For a long time he taught in universities. Many highly-qualified microbiologists were trained by him. Some of his students occupy directorships at the present time.

E. N. Mishustin takes an active part in public life. He is a member of the biological sciences section of the Academy of Sciences, USSR, an acting editor of the journal "Izvestiya Akad. Nauk SSSR, biological series," a member of the editorial board of the journal "Mikrobiologiya," a plenary member of VAK, chairman of the biology section of the All-Union Society of Soil Scientists, and chairman of the biology section of the Moscow City Society for the Propagation of Political and Scientific Knowledge.

Evgenii Nikolaevich Mishustin has a many-sided and productive scientific and public life. We wish him health, happiness, and many years of productive work for the good of our native land and for science.

SAMUIL L'VOVICH SOBOL'

A. I. Metelkin

Translated from Mikrobiologiya, Vol. 30, No. 3, p. 573, May-June, 1961

Soviet History of Natural Science mourns the loss of one of its best-known workers, Professor Samuil L'vovich Sobol', who died December 1, 1960, after a serious attack of grippe. His loss is particularly lamented by those scientific fields which use the microscope daily in their laboratory work.

S. L. Sobol' was born August 25, 1893, into the working-class family of an Odessa "watch-maker"; after finishing high school in Tbilisi, he entered Novorossiiskii University (in Odessa), where he studied in two departments at once—the Natural Science Division of the Department of Physics and Mathematics and the Philosophical Sciences Division of the Department of History and Philology. With the aid of this theoretical training, the young man was able to mark out clearly the interesting path that his productive work would follow for the rest of his life. Specializing in comparative histology, he taught the General Biology course at the Medical Institute and several other Odessa educational institutions. In 1922, he moved to Moscow, where he worked for a long time in the editorial office of the "Big Soviet Encyclopedia"; in 1929, he went to work for the Communist Academy (affiliated with the USSR Central Executive Comittee), in the History of Natural Science section, until 1938, when he started to work in the Division of Biological Sciences of the USSR Academy of Sciences; his last post was Senior Scientific Worker in the Academy's Institute of Natural Science (known since 1953 as the Institute of the History of Natural Science and Technics). An expert on Charles Darwin, S. L. Sobol' was one of the editors of the Soviet edition of Darwin's complete works.

Samuil L'vovich's outstanding achievement, however, was his organization of the collection and study of old microscopes used in national science from the very beginning of laboratory research work in Russian Natural Science. With his characteristic energy, he collected in a short time over 1,000 old-fashioned microscopes and other optical instruments and original microscopic preparations from the laboratories of our universities and hospitals and from individuals, organizing in this way our first "Cabinet of the History of Microscopes and Microscope Technique" in 1929 at the Communist Academy, where he began to study systematically the history of the use of the microscope in old Russia. Unfortunately, dissolution of this academy and the subsequent Great Patriotic War interrupted the Cabinet's work with its rich collection of extremely valuable historical objects. However, Samuil L'vovich continued to study the history of the Russian micriscope, publishing a whole series of different articles on the subject before completing and successfully defending his Doctor's dissertation. The latter, a lengthy monograph titled "The History of the Microscope and Microscopic Research in Russia during the Eighteenth Century," was published by the USSR Academy of Sciences (Moscow-Leningrad, 1949, 606 p.). For this remarkable and highly esteemed work, the author received in 1950 the honorary title of Stalin Prize Laureate. In June, 1946, the Presidium of the USSR Academy of Sciences passed a resolution initiated by an idea of S. L. Sobol''s to organize a History of Microscopy Division at the Institute of the History of Natural Science. I helped in the work of restoring the ancient microscopes, and, as I write these sad lines, memories of that time urge me to mention the late Samuil L'vovich's burning enthusiasm about this, the world's second greatest collection of old-fashioned optical instruments (the English collection being the greatest), and his profound conviction of its unique value to Russian science.

The monograph mentioned above is a serious and original research work which gives not only a systematic history of how the microscope was gradually developed in various fields of natural science, but also a commentary on contemporary opinion of scientific thought processes in eighteenth century Russia. This printed work describes the persistent struggle of Russian materialistic thought with the reactionary views of "imported" foreign scientists. The priority of Russian workers in the use of the microscope in several fields two centuries ago and that of our master opticians in its manufacture and technical perfection are demonstrated by the many conclusive facts set forth in this book. Without exaggeration, one can say that the late author resuscitated the memory of such eighteenth century microscope workers as M. M. Terekhovskii, A. M. Shumlyanskii, D. S. Samoilovich, A. T. Bolotov and many others by demonstrating the historical importance of their investigations. The author's profound study of scientific instruments, microscopic preparations and old records going back to the time of Peter's "Kunstkammer" enabled him to bring this historical work up to the beginning of the nineteenth century. How sad it is that this great researcher, true patriot and wonderful human being, Samuil L'vovich, could not live to complete his research on the nineteenth century. The name of this outstanding worker will long remain bright in the annals of our optical industry and national science.

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci.-Tech. Press
GOST	All-Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for Experimental Morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

Soviet Journals Available in Cover-to-Cover Translation

ABBREVIATION	RUSSIAN TITLE	TITLE OF TRANSLATION	PUBLISHER	TRANSLATION BEGAN Vol.	Issue	Year
AÉ	Atomnaya énergiya	Soviet Journal of Atomic Energy	Consultants Bureau	1	1	1956
Akust. zh.	Akusticheskii zhurnal	Soviet Physics – Acoustics	American Institute of Physics	1	1	1955
	Antibiotiki	Antibiotics	Consultants Bureau	4	1	1959
Astr(on). zh(urn).	Astronomicheskii zhurnal	Soviet Astronomy–AJ	American Institute of Physics	34	1	1957
Avto(mat). svarka	Avtomaticheskaya svarka	Automatic Welding	British Welding Research Association (London)		1	1959
	Avtomatika i Telemekhanika	Automation and Remote Control	Instrument Society of America	27	1	1956
	Biofizika	Biophysics	National Institutes of Health*		1	1957
	Biokhimiya	Biochemistry	Consultants Bureau	21	1	1956
Byull. éksp(erim). biol. i med.	Byulleten' éksperimental'noi biologii i meditsiny	Bulletin of Experimental Biology and Medicine	Consultants Bureau	41	1	1959
DAN (SSSR) } Dokl(ady) AN SSSR }	Doklady Akademii Nauk SSSR	The translation of this journal is published in sections, as follows:				
		Life Sciences: Doklady Biochemistry Section	American Institute of Biological Sciences	106	1	1956
		Life Sciences: Doklady Biological Sciences Sections (Includes: Anatomy, biophysics, cytology, ecology, embryology, endocrinology, evolutionary morphology, genetics, histology, hydrobiology microbiology, morphology, parasitology, physiology, zoology sections)	American Institute of Biological Sciences	112	1	1957
		Life Sciences: Doklady Botanical Sciences Sections (Includes: Botany, phytopathology, plant anatomy, plant ecology, plant embryology, plant physiology, plant morphology sections)	American Institute of Biological Sciences	112	1	1957
		Chemical Sciences: Proceedings of the Academy of Sciences of the USSR, Section: Chemical Technology	Consultants Bureau	106	1	1956
		Chemical Sciences: Proceedings of the Academy of Sciences of the USSR, Section: Chemistry	Consultants Bureau	106	1	1956
		Chemical Sciences: Proceedings of the Academy of Sciences of the USSR, Section: Physical Chemistry	Consultants Bureau	112	1	1957
		Earth Sciences: Doklady Earth Sciences Sections (Includes: Geochemistry, geology, geophysics, hydrogeology, mineralogy, paleontology, petrography, permafrost sections)	American Geological Institute	124	1	1959
		Earth Sciences: Proceedings of the Academy of Sciences of the USSR, Section: Geochemistry	Consultants Bureau	106-123	1-6	1957-1958
		Earth Sciences: Proceedings of the Academy of Sciences of the USSR, Sections: Geology	Consultants Bureau	106-123	1-6	1957-1958
		Mathematics: Doklady Soviet Mathematics	The American Mathematics Society	131	1	1961
		Physics: Soviet Physics–Doklady (Includes: Aerodynamics, astronomy, crystallography, cybernetics and control theory, electrical engineering, energetics, fluid mechanics, heat engineering, hydraulics, mathematical physics, mechanics, physics, technical physics, theory of elasticity sections)	American Institute of Physics	106	1	1956
		Physics: Proceedings of the Academy of Sciences of the USSR, Applied Physics Sections (does not include mathematical physics or physics sections)	Consultants Bureau	106-117	1	1956-1957
Derevoobrabat. prom-st'.	Derevoobrabatyvayushchaya promyshlennost'	Wood Processing Industry	Timber Development Association (London)		9	1959
	Élecktrosvyaz	Telecommunications	Massachusetts Institute of Technology*		1	1957
Éntom(ol). oboz(renie)	Éntomologicheskoe obozrenie	Entomological Review	American Institute of Biological Sciences	38	1	1959
Farmakol. (i) toksikol(ogiya)	Farmakologiya i toksikologiya	Pharmacology and Toxicology	Consultants Bureau	20	1	1957
FMM	Fizika metallov i metallovedenie	Physics of Metals and Metallography	Acta Metallurgica*	5	1	1957
Fiziol. zhurn. SSSR (im. Sechenova)	Fiziologicheskii zhurnal im. I. M. Sechenova	Sechenov Physiological Journal USSR	National Institutes of Health*		1	1957
Fiziol(ogiya) rast.	Fiziologiya rastenii	Plant Physiology	American Institute of Biological Sciences	4	1	1957
	Geokhimiya	Geochemistry	The Geochemical Society		1	1958
FTT	Fizika tverdogo tela	Soviet Physics–Solid State	American Institute of Physics	1	1	1959
Izmerit. tekh(nika)	Izmeritel'naya tekhnika	Measurement Techniques	Instrument Society of America		1	1959
Izv. AN SSSR, O(td). Kh(im). N(auk)	Izvestiya Akademii Nauk SSSR: Otdelenie khimicheskikh nauk	Bulletin of the Academy of Sciences of the USSR: Division of Chemical Sciences	Consultants Bureau		1	1952

continued

Izv. AN SSSR, O(td). T(ekhn). N(auk): Met(all). i top.	(see Met. i top.)				
Izv. AN SSSR Ser. fiz(ich).	Izvestiya Akademii Nauk SSSR: Seriya fizicheskaya	Bulletin of the Academy of Sciences of the USSR: Physical Series	Columbia Technical Translations	1	1954
Izv. AN SSSR Ser. geofiz.	Izvestiya Akademii Nauk SSSR: Seriya geofizicheskaya	Bulletin (Izvestiya) of the Academy of Sciences USSR: Geophysics Series	American Geophysical Union	1	1954
Izv. AN SSSR Ser. geol.	Izvestiya Akademii Nauk SSSR: Seriya geologicheskaya	Izvestiya of the Academy of Sciences of the USSR: Geologic Series	American Geological Institute	1	1958
Kauch. i rez.	Kauchuk i rezina	Soviet Rubber Technology	Research Association of British Rubber Manufacturers	18 1	1959
	Kinetika i kataliz	Kinetics and Catalysis	Consultants Bureau	1 3	1960
	Koks i khimiya	Coke and Chemistry USSR	Coal Tar Research Association (Leeds, England)	1	1958
Kolloidn. zh(urn).	Kolloidnyi zhurnal	Colloid Journal	Consultants Bureau	14 1	1952
	Kristallografiya	Soviet Physics – Crystallography	American Institute of Physics	2 1	1957
Metalov. i term. obrabot. metal.	Metallovedenie i termicheskaya obrabotka metallov	Metal Science and Heat Treatment of Metals	Acta Metallurgica	6 1	1958
	Metallurg	Metallurgist	Acta Metallurgica	1	1957
Met. i top.	Metallurgiya i topliva	Russian Metallurgy and Fuels	Eagle Technical Publications	1	1960
Mikrobiol.	Mikrobiologiya	Microbiology	American Institute of Biological Sciences	26 1	1957
OS	Optika i spektroskopiya	Optics and Spectroscopy	American Institute of Physics	6	1959
	Pochvovedenie	Soviet Soil Science	American Institute of Biological Sciences	1	1958
	Priborostroenie	Instrument Construction	British Scientific Instrument Research Association	1	1959
Pribory i tekhn. éks(perimenta)	Pribory i tekhnika éksperimenta	Instruments and Experimental Techniques	Instrument Society of America	1	1957
Prikl. matem. i mekh.	Prikladnaya matematika i mekhanika	Applied Mathematics and Mechanics	American Society of Mechanical Engineers	1	1958
PTÉ	(see Pribory i tekhn. éks.)				
	Problemy Severa	Problems of the North	National Research Council of Canada		
Radiotekh.	Radiotekhnika	Radio Engineering	Massachusetts Institute of Technology*	12 1	1957
Radiotekh. i élektronika	Radiotekhnika i élektronika	Radio Engineering and Electronics	Massachusetts Institute of Technology*	2 1	1957
	Stanki i instrument	Machines and Tooling	Production Engineering Research Assoc.	1	1959
	Stal'	Stal (In English)	Iron and Steel Institute	1	1959
Stek. i keram.	Steklo i keramika	Glass and Ceramics	Consultants Bureau	13 1	1956
Svaroch. proiz-vo	Svarochnoe proizvodstvo	Welding Production	British Welding Research Association	4	1959
Teor. veroyat. i prim.	Teoriya veroyatnostei i ee primenenie	Theory of Probability and Its Applications	Society for Industrial and Applied Mathematics	1	1956
Tsvet. Metally	Tsvetnye metally	Nonferrous Metals	Primary Sources	1	1960
UFN	Uspekhi fizicheskikh Nauk	Soviet Physics – Uspekhi (partial translation)	American Institute of Physics	66 1	1958
UKh	Uspekhi khimii	Russian Chemical Reviews	The Chemical Society (London)	1	1960
UMN	Uspekhi matematicheskikh nauk	Russian Mathematical Surveys	London Mathematical Society	15 1	1960
Usp. fiz. nauk	(see UFN)				
Usp. khim(ii)	(see UKh)				
Usp. matem. nauk	(see UMN)				
Usp. sovr. biol.	Uspekhi sovremennoi biologii	Russian Review of Biology	Oliver and Boyd	48	1959
Vest. mashinostroeniya	Vestnik mashinostroeniya	Russian Engineering Journal	Production Engineering Research Assoc.	4	1959
Vop. gem. i per. krovi	Voprosy gematologii i perelivaniya krovi	Problems of Hematology and Blood Transfusion	National Institutes of Health*	1	1957
Vop. onk.	Voprosy onkologii	Problems of Oncology	National Institutes of Health*	1	1957
Vop. virusol.	Voprosy virusologii	Problems of Virology	National Institutes of Health*	1	1957
Zav(odsk). lab(oratoriya)	Zavodskaya laboratoriya	Industrial Laboratory	Instrument Society of America	25 1	1959
ZhAKh Zh. anal(it). khimii	Zhurnal analiticheskoi khimii	Journal of Analytical Chemistry USSR	Consultants Bureau	7 1	1952
ZhETF Zh. éksperim. i teor. fiz.	Zhurnal éksperimental'noi i teoreticheskoi fiziki	Soviet Physics—JETP	American Institute of Physics	28 1	1955
ZhFKh Zh. fiz. khimii	Zhurnal fizicheskoi khimii	Russian Journal of Physical Chemistry	The Chemical Society (London)	7	1959
ZhMÉI Zh(urn). mikrobiol. épidemiol. i immunobiol.	Zhurnal mikrobiologii, épidemiologii i immunobiologii	Journal of Microbiology, Epidemiology and Immunobiology	National Institutes of Health*	1	1957
ZhNKh Zh(urn). neorgan(ich). khim(ii)	Zhurnal neorganicheskoi khimii	The Russian Journal of Inorganic Chemistry	The Chemical Society (London)	1	1959
ZhOKh Zh(urn). obshch(ei) khimii	Zhurnal obshchei khimii	Journal of General Chemistry USSR	Consultants Bureau	19 1	1949
ZhPKh Zh(urn). prikl. khimii	Zhurnal prikladnoi khimii	Journal of Applied Chemistry USSR	Consultants Bureau	23 1	1950
ZhSKh Zh(urn). strukt. khimii	Zhurnal strukturnoi khimii	Journal of Structural Chemistry	Consultants Bureau	1 1	1960
ZhTF Zh(urn). tekhn. fiz.	Zhurnal teknicheskoi fiziki	Soviet Physics—Technical Physics	American Institute of Physics	26 1	1956
Zh(urn). vyssh. nervn. deyat. (im. Pavlova)	Zhurnal vysshei nervnoi deyatel'nosti (im. I. P. Pavlova)	Pavlov Journal of Higher Nervous Activity	National Institutes of Health*	1	1958

*Sponsoring organization. Translation through 1960 issues is a publication of Pergamon Press.

AIBS Russian Monograph Translations

The AIBS is in the process of expanding its Russian Translations Program extensively. Funds to subsidize translation and publication of important Russian literature in biology have been obtained from the National Science Foundation, as part of a larger program to encourage the exchange of scientific information between the two countries. The following monographs have been published:

Origins of Angiospermous Plants. *By A. L. Takhtajan.*
Edited by G. Ledyard Stebbins. Translated by Olga H. Gankin.
68 pp. $3.00, individuals and industrial libraries (U.S.A. and Canada);
$2.00, AIBS members and all other libraries; $.50 additional, foreign.

Essays on the Evolutionary Morphology of Plants. *By A. L. Takhtajan.*
Edited by G. Ledyard Stebbins. Translated by Olga H. Gankin.
144 pp. $5.00, individuals and industrial libraries (U.S.A. and Canada);
$4.00, AIBS members and all other libraries; $.50 additional, foreign.

Problems in the Classification of Antagonists of Actinomycetes. *By G. F. Gause.*
Edited by David Gottlieb. Translated by Fritz Danga.
$5.00, individuals and industrial libraries (U.S.A. and Canada);
$4.00, AIBS members and all other libraries; $.50 additional, foreign.

Arachnida. Vol. IV, No. 2. Fauna of the U.S.S.R. *By B. I. Pomerantzev.*
Edited by George Anastos. Translated by Alena Elbl.
$10.00, individuals and industrial libraries (U.S.A. and Canada);
$9.00, AIBS members and all other libraries; $1.00 additional, foreign.

Arachnoidea. Vol. VI, No. 1. Fauna of the U.S.S.R. *By A. A. Zachvatkin.*
Translated and edited by A. Ratcliffe and A. M. Hughes.
$10.00, individuals and industrial libraries (U.S.A. and Canada);
$9.00, AIBS members and all other libraries; $1.00 additional, foreign.

Marine Biology. Vol. XX, Trudy Institute of Oceanology. *Edited by B. N. Nikitin.*
$10.00, individuals and industrial libraries (U.S.A. and Canada);
$7.50, AIBS members and all other libraries; $1.00 additional, foreign.

Plants and X-rays. *By L. P. Breslavets.*
Editor of English edition: Arnold H. Sparrow.
$5.00, individuals and industrial libraries (U.S.A. and Canada);
$4.00, AIBS members and all other libraries; $.50 additional, foreign.

Wintering of Plants. *By I. M. Vasiliev.*
Editor of English edition: Jacob Levitt.
$9.00, individuals and industrial libraries (U.S.A. and Canada);
$8.00, AIBS members and all other libraries; $.50 additional, foreign.

All orders and subscriptions should be placed with:

AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES
2000 P St., N.W. Washington 6, D. C.

ЖУРНАЛЫ БИОЛОГИИ

A chance to compare and utilize.....

ENGLISH TRANSLATIONS OF RUSSIAN BIOLOGICAL JOURNALS

The American Institute of Biological Sciences, under a grant from the National Science Foundation, is currently translating and publishing the following seven Russian research journals in biology.

DOKLADY
BIOLOGICAL SCIENCES SECTIONS

Dr. Charles C. Davis, *Advisory Editor*

6 issues per year. First issues published: 1957, Vols. 112-117. Current issues published: 1961, Vols. 136-141.

This is a translation of all papers in the Biological Sciences Sections of the Proceedings of the Academy of Sciences of the USSR. It includes sections in many areas of biology, among which are the following: biophysics, cytology, embryology, experimental morphology, genetics, histology, hydrobiology, microbiology, morphology, physiology, and zoology.

YEARLY SUBSCRIPTIONS: $20.00, individuals and industrial libraries (U.S. & Can.); $15.00, AIBS members and all other libraries; $2.50 additional to each price, foreign. SINGLE COPIES: $4.00.

DOKLADY
BOTANICAL SCIENCES SECTIONS

Dr. Helen P. Sorokin, *Advisory Editor*

6 issues per year. First issues published: 1957, Vols. 112-117. Current issues published: 1961, Vols. 136-141.

This journal is a translation of all papers in the Botanical Sciences Sections of the Proceedings of the Academy of Sciences of the USSR. It includes sections on botany, plant anatomy, plant embryology, plant morphology, and plant physiology.

YEARLY SUBSCRIPTIONS: $7.50, individuals and industrial libraries (U.S. & Can.); $5.75, AIBS members and all other libraries; $1.50 additional to each price, foreign. SINGLE COPIES: $1.50.

DOKLADY
BIOCHEMISTRY SECTION

Dr. Jakob A. Stekol, *Advisory Editor*

6 issues per year. First issues published: 1957, Vols. 112-117. Current issues published: 1961, Vols. 136-141.

This is a translation of all papers in the Biochemistry Section of the Proceedings of the Academy of Sciences of the USSR. These papers deal with all phases of the chemistry of plant and animal life, at both the biological and physiological levels. This translation contains a list of abbreviations most frequently encountered in Russian Bio-sciences literature, as well as a reference list of Russian journals that are frequently cited.

YEARLY SUBSCRIPTIONS: $15.00, individuals and industrial libraries (U.S. & Can.); $10.00, AIBS members and all other libraries; $3.00 additional to each price, foreign. SINGLE COPIES: $4.50.

PLANT PHYSIOLOGY
(FIZIOLOGIYA RASTENII)

Dr. Arthur W. Galston, *Advisory Editor*
Dr. Bruce A. Bonner, *Asst. Advisory Ed.*

6 issues per year. First issues published: 1957, Vol. 4, Nos. 1-6. Current issues published: 1961, Vol. 7, Nos. 1-6.

This journal publishes original studies of an experimental nature on various problems of the physiology of plants, reviews, critical articles, and discussions of major problems in this field. It has special sections dealing with methods of experimental research and the application of finished scientific research to current agricultural practice.

YEARLY SUBSCRIPTIONS: $15.00, individuals and industrial libraries (U.S. & Can.); $11.00, AIBS members and all other libraries; $2.00 additional to each price, foreign. SINGLE COPIES: $3.00.

MICROBIOLOGY
(MIKROBIOLOGIYA)

Dr. J. R. Porter, *Advisory Editor*

6 issues per year. First issues published: 1957, Vol. 26, Nos. 1-6. Current issues published: 1961, Vol. 30, Nos. 1-6.

This journal deals with problems of general microbiology (systematics, morphology, physiology, and variability of microorganisms), agricultural microbiology (microbiology of soils and fodder crops, bacterial fertilizers), technical microbiology, the microbiology of foods, and the microbiology of water reservoirs. This journal also contains sections on the history of science, book reviews, and current events in the field of microbiology, as well as the general articles in this field.

YEARLY SUBSCRIPTIONS: $20.00, individuals and industrial libraries (U.S. & Can.); $15.00, AIBS members and all other libraries; $2.50 additional to each price, foreign. SINGLE COPIES: $4.00.

ENTOMOLOGICAL REVIEW
(ENTOMOLOGICHESKOE OBOZRENIE)

Dr. C. F. W. Muesebeck, *Advisory Editor*

4 issues per year. First issues published: 1958, Vol. 37, Nos. 1-4. Current issues published: 1961, Vol. 40, Nos. 1-4.

Very few entomologists would not find something of special interest to them in any one issue of this periodical. It deals with theoretical and practical problems in the field of entomology and their significance for the national economy. It contains articles on all branches of entomology and devotes particular attention to problems connected with the theoretical basis of agriculture and public health service.

YEARLY SUBSCRIPTIONS: $25.00, individuals and industrial libraries (U.S. & Can.); $12.00, AIBS members and all other libraries; $3.00 additional to each price, foreign. SINGLE COPIES: $7.50.

SOVIET SOIL SCIENCE
(POCHVOVEDENIYE)

Dr. A. P. Mazurak, *Editor*

12 issues per year. First issues published: 1958. Current issues published: Jan.-Dec. 1960.

This is a translation of the Russian journal, Pochvovedeniye, edited by Dr. A. P. Mazurak of the University of Nebraska. It publishes research done at various institutes in the fields of soil chemistry, soil physics, soil microbiology, soil fertility, soil genesis and classification, soil conservation, irrigation, and related aspects of soil science.

YEARLY SUBSCRIPTIONS: $40.00, individuals and industrial libraries (U.S. & Can.); $20.00, AIBS members and all other libraries; $3.00 additional to each price, foreign. SINGLE COPIES: $4.50.

All orders and subscriptions should be placed with:

AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES
2000 P Street, N.W. Washington 6, D.C.